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The consequences of Solzhenitsyn

So Solzhenitsyn has been deprived of his citizenship and unceremoniously bundled out of his fatherland in the same way that Lenin was nearly seventy years ago. We have to be grateful, presumably, that the Soviet government did not choose to exact a more loathsome penalty, such as banishment to a work camp in Siberia. We must also be thankful that his family are to be allowed to follow. The fact that worse has not befallen him physically should not, however, diminish our sense of shock that charges of treason should be brought against a man who, from a standpoint of undoubted patriotism, chooses to criticise his country. For if not patriotic (and heaven knows it shines through his novels) why did he not leave his homeland years ago for the comforts of the western world?

He knew that his voice was essentially a Russian one, and he must have known that banishment was the only sanction which could wound his spirit.

The action that the Soviet government took shows the way in which it had allowed itself to become boxed in by one man. In the end, by taking Solzhenitsyn so desperately seriously, it found itself having to use all the state machinery to destroy one single citizen. Yet, at any time in the last year, there was the opportunity to bow out of the conflict and let him make all the running himself. After all, his writings were little known in the Soviet Union, so there were slim prospects that he would be able to gather together any sort of populist following among the Russian public that could ever form a physical threat to internal security: not that he had ever given any indications of a leaning in this direction. Furthermore, there had been sharply divided opinion in the west on the efficacy of his many messages. Many have accepted his observation on the situation in the Soviet Union without too much hesitation, but fewer have been able to go along with his conclusions for political remedy—particularly his calls on western countries to take a tough line in their dealings with the Soviet government.

The way had thus been open simply to isolating and ignoring Solzhenitsyn, merely on the grounds that he had a small constituency at home, and was not being effectively harmful to Soviet interests abroad. Instead, the persecution has undoubtedly done this very harm. A most effective way to create a majority is to attempt to destroy a minority by heavy handed means: many who up to the present have been prepared to accept Solzhenitsyn as a great writer but have thought him naive politically are now ready to take him, or rather the political system that he exposes, much more seriously.

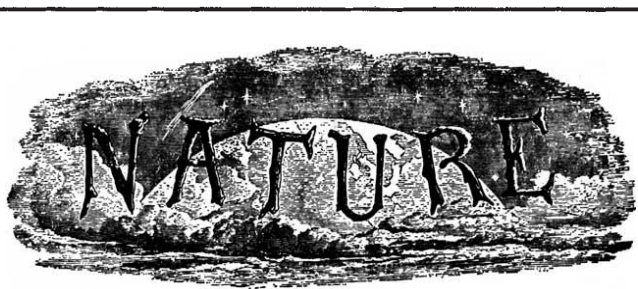
And is Sakharov next?

Once more we shall be swept up in a debate amongst western intellectuals about institutional and individual attitudes to the Soviet Union. If the Solzhenitsyn incident teaches us one thing, it is that ways of thinking in East and West are utterly different on questions of political freedom. It seems that a Hungary, a Czechoslovakia, a Solzhenitsyn is periodically necessary to remind us of this gulf, although, lest we become too arrogant it must be said that there are many calamities attributable to the western political system. Nevertheless, the point is that the difference in character is not something which is likely to be changed by the chilling of diplomatic relationships, by bitterness at Geneva or by the breaking of ties between learned institutions. The official links are all operated by men with seniority and a weight of tradition behind them. These men are unlikely to be susceptible to persuasion.

It is a change in human nature which is being sought by many westerners. Is it foolish and impertinent even to think of wreaking such a change? Probably not. Western attitudes are not what they were ten, a hundred or a thousand years ago, and there is no reason to believe that eastern attitudes cannot change likewise. Indeed, the relative lack of change in recent times makes the potential for future change greater.

This is unlikely to be effected at an institutional level—a parliament or learned body is rarely able to trigger a revolution in patterns of thought. It has to be done by allowing every possible channel of human contact to be exploited. What is needed now is not boycott but more flow, particularly amongst the young.

100 years ago



A Lecture Experiment

THE condensation of liquid in the form of vapour into minute globules, and the production of a shower of rain, may be very well illustrated for class purposes in the following manner:—

Place about an ounce of Canada balsam in a Florence flask, and let it boil. At the top of the flask clouds of globules of turpentine will be seen hovering about, altering in shape very much like sky-clouds, and the globules are large enough to be visible by the naked eye. If a cold glass rod be gradually introduced into the flask, these clouds may be made to descend in showers. By the adaptation of a lime-light the whole process could be shown on a screen.

LAWSON TAIT

From *Nature*, 9, 323, February 26, 1874.



international news

FDA halts scheme to combat anaemia

Colin Norman, Washington

A CONTROVERSIAL scheme to provide what amounts to preventive medicine through supermarkets and grocery stores has been called off at the last moment by the US Food and Drug Administration (FDA). The scheme involves adding large helpings of iron to most of the bread baked and sold in the United States, in an effort to combat iron deficiency anaemia.

Under pressure from influential medical and nutrition organisations, the FDA issued a regulation in October last year which would have tripled the amount of iron in so-called 'enriched' bread and flour. The regulation, which has the force of law, was due to be brought into effect on April 15, and most bakeries have already made plans to implement it. But a barrage of complaints from individual physicians, concerned about possible danger to people suffering from rare blood disorders, led the FDA to announce last week that it will turn the whole matter over to a public hearing on April 1. The regulation will not be enforced until the safety question has been cleared up, the FDA said.

The idea of adding iron to bread and flour has influential support from the American Medical Association, the Food and Nutrition Board of the National Academy of Sciences, the White House Conference on Nutrition, and virtually every national nutrition association. With such an impressive pedigree, and a goal with which few would disagree, why has the scheme met with so much opposition?

The dispute is, on the surface, simply a straightforward disagreement among scientists and physicians over the interpretation of facts and the results of various clinical and epidemiological studies. But it also touches on some fundamental principles underlying the FDA's approach to regulating the food industry, and it calls into question the federal government's role in trying to improve the nutritional standard of the American diet. In any case, it is an interesting case study of regulatory decision-making.

The idea of fortifying basic foods

with iron compounds gained impetus in the 1960s from studies which indicated that anaemia is widespread in the United States, particularly among women and children from low income groups. Since bread is the most widely consumed processed food, it became the obvious candidate for a fortification programme designed to increase iron intake, and in 1969 the idea was floated by the White House Conference on Nutrition as one of its many recommendations for improving the health of the American public.

A year after the White House Conference published its report, the idea was converted into a formal proposal from the American Bakers Association and the Millers National Federation, the baking industry's trade associations. They asked that the legally required amounts of iron in so-called enriched flour and bakery products be increased significantly from their present levels.

The FDA handled the proposal with some caution, first by turning it over to the American Medical Association for advice, and then by coming up with a proposal of its own. It is the FDA's proposal which was issued in final form last October, and which will be the subject of public hearings in April.

In short, the proposal would require that, in order to bear the label 'enriched', all flour sold in the United States must contain 40 mg of iron per pound, and enriched bread must similarly contain 25 mg of iron per pound. According to the FDA, some two thirds of all bakery products now consumed in the United States are enriched. And, in view of the rapid progress of the fortification bandwagon during the past few years—a phenomenon which has turned most breakfast foods into little more than high-calorie vitamin pills—there is a strong commercial incentive for bakeries to produce goods which can bear the 'enriched' label.

If the proposal is adopted and made a legal requirement, it will not be the first time that basic foods have been loaded with extra nutrients. For many years, vitamin D has been added to milk and iodine has been added to salt, and even iron has, for the past 30 years, been baked into bread. But the proposal represents a radically new policy. The present requirement is that enriched bread should contain between 8 mg and 12.5 mg of iron per pound, simply to ensure that the iron lost dur-

ing milling of white flour is replaced; it is a policy which is followed in most industrialised countries. The proposed levels, however, represent a deliberate attempt to increase the average American's daily intake of iron.

According to the FDA, even at the proposed levels, the amount of extra iron that would be included in the average diet would amount to less than 20% of the recommended daily requirement. Thus, the agency argued when it published its final regulation, the scheme can hardly be called 'super-market medication'. The proposal, the FDA said, strikes a balance between providing as much of the recommended daily allowance as possible, and ensuring that the risks to people suffering from blood disorders are kept to a minimum.

But critics of the scheme are not so sanguine. First, they have criticised the studies which led to the theory that anaemia is prevalent in the United States, and they have argued that there is need for more accurate information on the epidemiology of iron deficiency anaemia before such a fortification scheme is begun. Second, they have cited a sheaf of studies which cast doubt on the extent to which iron incorporated in bread is assimilated by man; again, they suggest that more information is needed before iron salts are loaded into bread. And they have also pointed out that nobody has yet managed to quantify the risks of increased iron intake to people suffering from such blood disorders as haemochromatosis, Cooley's anaemia and liver disease associated with alcoholism. In other words, even though the idea of fortifying bread with iron was first proposed more than five years ago, the chief argument being put up against the scheme is that it is premature.

As for the prevalence of anaemia, the FDA bases much of its case on a study carried out in the 1960s, called the Ten State Nutrition Survey. This study indicated widespread anaemia, particularly among people from lower income groups, and it also came up with the finding that the problem is not solely confined to women and children. The methods employed in the study have, however, been criticized by several haematologists, who have argued that the conclusions may be false, and also that, even if anaemia is as prevalent as the study indicates, inadequate iron is not the sole cause.

In response to such doubts, the FDA set in train a reappraisal of the literature, from which its experts concluded that "there is a strikingly high incidence of iron deficiency anaemia in many segments of the US population". They also concluded that the studies have "consistently shown higher anaemia prevalence rates among blacks compared to whites, in low income states compared to high income states, and in low socio-economic groups compared with groups higher in this regard". And those conclusions have been endorsed by virtually all the major medical associations in the United States.

But even if the studies are correct, would an iron fortification scheme help? Again, the FDA and the professional medical associations say that it would, and some individual physicians say that such a conclusion is not necessarily supported by the evidence. Writing in *Nutrition Today*, for example, Dr Maxwell Wintrobe, Professor of Medicine at the University of Utah, and a highly respected haematologist, points out that a number of studies have indicated that iron salts added to bread are not readily absorbed by the body. In particular, a study carried out in Wales by Dr. Peter Elwood, which involved two large communities, produced "no conclusive evidence in terms of an effect on circulating haemoglobin levels", Wintrobe asserts. Wintrobe also pointed out that the signs of anaemia picked up by the ten state survey may be put down to a variety of causes, and thus simply increasing dietary iron intake can only solve part of the problem.

The FDA, however, does not concede that the iron fortification scheme would be either unnecessary or ineffective. Dr Alexander Schmidt, Commissioner of the Food and Drug Administration, specifically stated last week when he announced that the proposal would be suspended, that "several tests, including measurements of iron serum, iron absorption, and iron binding capacity have been utilized to verify that low haematocrit and haemoglobin values observed in nutritional studies establishing the prevalence of anemia . . . are due to iron deficiency". Schmidt also cited two studies to show that man does utilize iron furnished by fortified bread. Thus, the FDA has announced that it will not consider further objections on those two points—the only recourse opponents have if the regulation is imposed is to go to court—and the hearings in April will thus be concerned solely with the narrow question of risk.

The hearing will thus be a scientific debate over the likely effects of increased iron intake on people suffering from storage diseases, but two other considerations are likely to arise. The first is that additional dietary iron could mask

the diagnosis of diseases which are usually signalled by the onset of anaemia. Cancer of the colon, which causes chronic internal bleeding, is the most frequently cited example. And second, it has recently been suggested that intake of additional quantities of iron could lead to the development of Parkinson's disease in some people, because the disease is associated with increased iron stores in the brain.

The hearings will thus be concerned solely with the risk side of the risk-benefit equation. That is, however, unlikely to satisfy many of the critics of the scheme, who are asking for a full inquiry into the philosophy behind the proposal, and the need for the federal government to require by law that iron be added to bread.

As Dr Wintrobe suggests, if poor nutrition is indeed the cause of iron deficiency anaemia in the United States, a more effective policy should aim at attacking the causes of poor nutrition. Such corrective measures as improving the economic status of lower income groups, reducing unemployment and lowering food prices would be more effective than "to cover our eyes, shut our minds, triple the dietary iron and hope for the best", he says.

British MPs do not like US reactor plan

John Hall

THE House of Commons Select Committee on Science and Technology, which has been examining the possible choices of nuclear reactor systems for Britain, has rushed out a report in record time in order not to be presented with a *fait accompli* by the government (Paper 145, HMSO., 1974; 14p). In the event Parliament was dissolved before any decision could be taken but the committee was nevertheless justifiably proud of its fast move. Its recommendation, which was pretty much a foregone conclusion, is that no proposal to build American light water reactors under licence should be approved on the basis of existing evidence. Questions of safety, costs and the effects of a purchase from the United States on Britain's own nuclear development programme are the bases of the committee's opinion.

To order light water reactors (LWRs) in the numbers proposed by the Central Electricity Generating Board (CEGB) would mean (the committee thinks) that apart from work on the fast breeder reactor Britain would virtually be abandoning its long established nuclear research and development effort.

As for the safety angle, the committee considers that in view of the conflicting opinion on the reliability of LWRs, it is for the proponents of light

Super bread and frozen hay

from our Soviet Correspondent

Two new nutritional advances have been announced by Soviet Scientists. A team working at Tashkent, in the cotton-growing Uzbek SSR has developed a method of extracting albumin from cotton seeds. The product, when added to dough, is said to result in bread identical in taste to the ordinary kind, but with a nutritional value equal to that of meat.

Meanwhile, down on the collective farm, an improved winter fodder is planned for livestock. Instead of the old-fashioned uneconomical hay, which loses between 20 and 40% of its nutritional value in the drying process, a new system of instant deep freezing of the mown grass at -17°C promises an all-year-round diet of fresh grass, with a carotene content twice that of hay.

water technology to prove its safety beyond reasonable doubt, rather than for their opponents to prove the contrary case. The point is of particular importance for a densely populated country like Britain, it says.

Evidence the committee heard on costing was so varied that members grew to be sceptical of any hard and fast quotation, regardless of its source. The report comments that no part of the evidence received on capital costs is directly comparable with any other part; this leads the committee to suspect that, unless there is a great deal of operating experience with the system in question, no one could guarantee that any given reactor system would prove cheaper than any other under actual operating conditions. In its opinion no sufficiently large nuclear reactor system has been operating for long enough to permit such confident estimates of installation and operating costs as those which the CEGB put forward to justify its preference for LWRs.

Common sense indicates, says the report, that until the high temperature gas cooled reactor (HTR) and the fast breeder reactor are available on a commercial basis the way forward should be to use one of the British nuclear technologies which is already proven, since this would be likely to satisfy the Nuclear Inspectorate without undue delay. Bearing this kind of consideration in mind, the committee notes in its report the enthusiasm of the South of Scotland Electricity Board (SSEB)

for the steam generating heavy water reactor (SGHWR).

The findings make the CEBG preference for American technology look distinctly shaky, since the mainstay of this preference is the cheapness and ready availability of the LWRs. Since the committee found costing estimates so wildly varied as to be unreliable, and the Nuclear Inspector's likely requirements so time consuming, the CEBG is left with the onus of offering better supported cash estimates, specifications less suspect to the Nuclear Inspectorate or an entirely new set of justifications for the hardware. At this stage of the game this does not look like a good exercise to be starting.

The CEBG had wanted to order nine twin-reactor stations, each of capacity 2,400–2,600 MW, between now and 1979, regardless of the choice of reactor. Mr A. E. Hawkins, Chairman of the CEBG, although denying that all of these orders would be for pressurised water reactors (PWRs) of American design, gave evidence to the committee which nevertheless suggests that this reactor would in fact account for the bulk of the CEBG's new nuclear plant if his plans were accepted. He had admitted, in the face of questioning, that LWRs of 1,200 to 1,300 MW are not yet in service, and therefore not proved.

The report comments that, since calculations up to 1980 are still being made on the basis of an annual growth in load of 4¼%, the committee is at a loss to see a pressing need for a decision to implement a programme of this size over such a long time scale, particularly as Mr Hawkins had said that the CEBG were, even before the current crisis, "very much exercised about the energy availability for the production of electricity in the 1980s and 1990s".

Unfortunately for the CEBG, Mr Hawkins suggested in 1972 that there was no great urgency about the choice of reactor system, and basing his calculations on a load growth of 5%, he said it was probable that only four new nuclear reactors would be required before 1982. Since these views were evidently mistaken, the committee remarks acidly, it will need much greater assurance that the CEBG's new plans are based on more valid assumptions.

Forward planning estimates and cost considerations apart, the American equipment simply will not come within a hundred miles of the English countryside if there is a really strong body of opinion that one day it may just give out and spill its contents, and the decision of the Nuclear Inspector is crucial to this issue. The report notes that although proponents of the PWR are convinced of its safety the Americans

themselves are sufficiently doubtful to be building a small PWR somewhere in the Idaho desert, where it is to be tested "almost to destruction" in order to determine whether or not it deserves a reputation for safety.

Reinforcing the doubt in this area, the report gives a prominent reminder of a memorandum submitted by Sir Alan Cottrell, the Chief Scientific Adviser to the Government, and one of Britain's most eminent metallurgists. Sir Alan's evidence read:

"Rapid fracture, from large cracks or defects in thick sections, is in principle possible in steel pressure vessels under operational conditions. In LWR vessels the estimated critical crack size for unstable growth is smaller than the wall thickness, so that the 'leak-before-break' safety feature is unavailable. In these circumstances, the security of an LWR vessel against fracture depends on the maintenance of rigorous manufacturing and quality control standards; and on thorough, effective and regularly repeated examination of the vessel by the ultrasonic crack-detection technique. The possible gradual growth of small cracks in highly stressed regions, by ageing and corrosion effects during service, needs further scientific investigation; as also does the effect of thermal shock from emergency cooling water in a loss-of-coolant incident."

The safety issue is all the more important since operating experience with PWRs of 500 MW and above is still very limited. Mr F. L. Tombs, Chairman of the SSEB, gave an estimate of about 20 reactor years of experience for large PWRs as against 200 reactor years of experience for British Magnox plant, and as recently as November Commissioner Doub of the United States Atomic Energy Commission was reported as saying that "while the technical feasibility and demonstration of the technology is a quarter of a century old the operating experience, especially with nuclear plants of the 800–1,000 MWe variety is still quite minimal".

The committee nicely sums up the impossible nature of the calculations involved in one distinctly world weary paragraph. In addition to the possibility of redesign to meet British safety requirements, they note any argument on the economics of reactor systems is influenced by variables including those in the following list:

"The sizes of the plants which are being compared, the dates to which the quotations refer, the estimates of availability and load factor for the systems in question, the extent to which wage escalation during construction and rises in fuel costs are taken into account, and the length of time which it takes from the first decision to purchase a station

to its entering commercial operation." There is no actual mention of Gordian knots or Augean stables, but there is more than a suggestion that that kind of job pales into insignificance.

Appointments down under

from a Correspondent

Two Americans have been appointed directors of the two largest, new research institutes in Australia. The Australian Institute of Marine Science (AIMS) will be headed by Professor Malvern Gilmartin, at present Professor of Biological Oceanography at the Hopkins Marine Station of Stanford University, and the Anglo-Australian Telescope (AAT) will be directed by Professor E. Joseph Wampler. The two will take up their duties later this year.

Four and a half years have elapsed from the initial announcement of the formation of AIMS by the previous Liberal-Country Party government of Australia. Negotiations over the site for AIMS between the Federal Labor Party government and the Country Party government of the State of Queensland have just been concluded with the designation of a site of 50 acres at Cape Cleveland, near Townsville in North Queensland.

Professor Gilmartin, whose advertised salary of \$A 19,148 is under review (it is at present the same as that for university professors), will have \$A 8 million to spend in the first five years. The institute is charged with researching the little known tropical and semi-tropical waters of Australia, notably in the regions of the Great Barrier Reef, the Coral Sea and the North Queensland coast.

The AAT is a 3.9 M (154-inch) optical reflector being built jointly by Britain and Australia. Professor Wampler's directorship is "for two years in the first instance", during which time he will be on leave from the Lick Observatory of the University of California where he has been an instrumental astronomer since 1967 with a reputation for playing a leading part in developing the image intensifier system.

The AAT is a highly instrumented and automated piece of equipment. It will probably turn out to be fractionally larger than the other big reflector in the southern hemisphere (3.9m), being built at the Cerro Tololo Observatory in Chile. The AAT is now in the final stages of construction on Siding Spring Mountain in north-western New South Wales. The commissioning astronomer, Professor S. C. Ben Gascoigne, of the Australian National University, is already integrating and calibrating all the Telescope's systems: he has ex-

pressed his enthusiasm for the high quality of the mirror which arrived recently from England.

The AAT will be fully operational by January 1975. The cost of establishing the AAT by then will have been \$A 16 million, shared equally between the United Kingdom and Australia. The AAT board has recently announced its estimates of the running costs: these will rise to \$A 1.74 million per annum in 1976-77 before steadying out at \$A 1.3 million per annum in following years. Professor Wampler's salary has not been announced.

In Australian scientific circles, there is much relief that these two projects in growth areas of Australian research can move into high gear at last, though there has been disappointment among local scientists that none of their number won either of these key posts.

The temporary nature of Professor Wampler's initial appointment underscores the uncertainty which has hung over the running of the AAT, as distinct from its design and construction which proceeded smoothly. There was much heartache over whether the Australian National University would have any special place in running the astronomical programme of the largest telescope on the site of their own Sliding Spring Observatory: the university lost on this point. The head of its Department of Astronomy, Professor Olin Eggen resigned from the AAT board last year. After protracted discussions, the AAT board, with equal representation of three Australian and three British nominees, did not settle on one of their own world class nationals as director. But, at least the AAT board has determined a policy of observing time being equally divided between British and Australian astronomers—under a tactful director of neutral nationality.

Oxfam solves a sewage problem

John Wilson

IN the hope of cutting down on infections caused by insanitary conditions in refugee camps Oxfam, the famine relief organisation, has commissioned original scientific research and is well on the way to developing an entirely novel kind of sewage disposal. Already it has arrived at a system which scores over conventional equipment by being cheap, easily transported, and capable of erection by unskilled labour. It may also result in the elimination of the organisms responsible for cholera and dysentery by containing sewage in anaerobic conditions.

Oxfam's interest in a sewage system was first kindled by its experience of the refugee camps in Bengal at the time

of the Indo-Pakistan war of 1971. Oxfam realised that most of its relief effort was often spent on the treatment of diseases resulting from insanitary conditions within the camps and that in many instances the preventive medicine used was not very effective in controlling these diseases—particularly cholera.

Containment of the sewage is a priority, so Oxfam looked for a suitable container. It considered using a portable fuel tank, developed by the Royal Air Force (RAF) as part of its back-up facilities for the harrier, and found that it would provide almost instantaneous anaerobic conditions. From this sprang the idea of destroying the pathogens anaerobically.

In the event, Oxfam found that the RAF tank had some shortcomings and decided instead to pass the effluent along a flexible tube about 100 feet long and 5 feet in diameter. This can be rolled up for transport and easily partitioned for the sedimentation of solid matter. Moreover, by sampling the effluent as it moves along the tube a ready estimate of the survival of pathogens can be made.

The water pollution research laboratory at Stevenage advised on the general design of a unit suitable for about 5,000 people, but there was one important fact that the Stevenage laboratory did not know. The survival time of the cholera vibrio in anaerobic sewage had never been determined, so it was not clear how long the sewage would have to be retained to eliminate the organism. Oxfam asked Mr B. Lloyd, of the University of Surrey, to study the retention problem.

With the assistance of a student, Mr Lloyd found that the survival of the organism depends on the temperature of the sludge. No cholera vibrios were found when the effluent was maintained at 37° C for 7 days but when the temperature was lowered to 25° C they took 12 days to disappear. In his final report to Oxfam, Mr Lloyd recommended that the sewage should be retained in anaerobic conditions for at least a fortnight. This piece of research cost Oxfam £50.

With a grant of £19,000 from the Leverhulme Trust, Oxfam next set about trying the system under operational conditions. They sent a microbiologist and two engineers to Bangladesh at the beginning of the year and on the evidence of their visit it is likely that there will be a large scale test project at a Bihari refugee camp in Muhamadpur, Dacca, in the near future. An extra £6,000 is needed before the plan can go ahead, but there is a suggestion that the government may come forward with the money. With the evidence of such enterprise on the part of a registered charity, it can scarcely do otherwise.

Setback for space science

Colin Norman, Washington

A LAUNCH vehicle designed to be NASA's chief workhorse for planetary flights in the 1970s, had to be blown up during its test flight last week. The rocket, a Titan-Centaur, was destroyed over the Atlantic when the Centaur stage failed to ignite. A board of inquiry has been set up to try to determine the cause of the failure, but from preliminary data, it seems that a boost pump failed to supply necessary pressure to the Centaur engines.

The episode has caused some consternation among planetary scientists, because the Titan-Centaur is designed to launch six important missions in the next few years—two Helios spacecraft to study the Sun, two Viking missions to Mars, and two Mariner spacecraft on a flyby mission around Jupiter and Saturn. If the failure is deemed serious enough to require a second test mission before the launch vehicle is entrusted with an expensive spacecraft, some of those missions may have to be delayed.

NASA officials last week were hopeful, however, that a second test flight will not be necessary, and that the first Helios launch can take place on schedule in September. According to Mr Joseph Mahon, director of NASA's launch vehicle and propulsion programmes, the flight provided much valuable information as far as it went, and many pieces of hardware specifically under test functioned perfectly. The guidance system, for example, which is located in the Centaur stage took the Titan stage on the correct course, and the shroud—the only major piece of equipment that had not been flown before on other rockets—jettisoned successfully.

The flight also provided data on the environment in the spacecraft section as it went through the lower reaches of the atmosphere, which is essential for the planning of future missions. Thus Mahon predicted that if the board of inquiry can pinpoint the failure exactly, then "we can launch Helios with a 99.9% certainty that it will function properly".

The failure itself occurred some eight minutes into the flight, as the vehicle was passing out of range of the Kennedy Space Center's tracking equipment. Thus it was not possible to tell with any certainty exactly what went wrong until data were relayed back from tracking stations further down line.

Apart from the Centaur rocket itself, its payload, consisting of a model of the Viking lander, and a spacecraft designed to measure the effect of charged particles on solar cells, was also blown to pieces.

Christmas prize quiz solution

THE best answer to the *Nature* Christmas Prize Quiz (246, 385; 1973) came from Nicholas Kennedy of the Max-Planck-Institut Für Molekulare Genetik, who wins the prize of a year's subscription to *Nature*.

The correct solution is as follows, with Kennedy's answers in parentheses where appropriate:

1 High flux reactor: France and Germany. 2 The White House. 3 Zhores Medvedev. 4 Lynden-Bell and Hoyle, now at Manchester. 5 Glaciation on Mars. 6 Advisory board for the research councils. 7 Computer printout from an aggressive game developed by Maynard-Smith and Price. 8 Britain and the Netherlands. 9 Appointed Chairman (*sic*) of Atomic Energy Commission. 10 Michael Heseltine misled the house about Hovertrain's fate. 11 Lord Snow (John Maddox). 12 Always. 13 J. Watson speaking of cuts in research funds. 14 six. 15 Sir Arnold Weinstock. 16 Japan. 17 Heimaey. 18 Hovertrain scrapped. 19 Motor car manufacturers (but not people who breathe air). 20 From $Z = 2.9$ to $Z = 3.4$. 21 5.9×10^6 . 22 Sakharov. 23 2030 AD. 24 Six and three. 25 S.F. Edwards. 26 Dinosaurs. 27 747 members of the Royal Society (the Elephant and Castle Bingo Club). 28 Stones, Tree trunks and large rocks. 29 Aigrain, Paris, MIT. 30 A Council for Science and Society. 31 Intraplate earthquakes. 32 Allotting money for sortie-lab. 33 Comité Recherche Européenne Science et Technologie: selling price increase tax (Society for the Prevention of Inane Tests). 34 International Union of Pure and Applied Physics: Voronel was not allowed to attend meetings in Amsterdam and Moscow. 35 Letcombe Laboratory. 36 2.9 M.Y. 38 Margaret rejoined Geoffrey in California. 39 Cottrell. 40 January 2 to 5, 1974.

An innocent abroad: science in London

John Gribbin

JUST how badly is the present series of crises affecting that most important of scientific traditions, the symposium or seminar? Without the cross pollination provided by these meetings, science as we know it would be impossible; learned societies are still managing to meet regularly, but not without difficulties,

as I discovered on a recent visit to the Scientific Societies Lecture Theatre at Savile Row in London. To be fair, all the happenings of that eventful afternoon are unlikely to coincide often—but they do provide a potted guide to the hazards of attending scientific meetings in February 1974.

Everything began well, and it was a relief to leave the candle-lit *Nature* offices, even if only for the slightly less unrelieved gloom of the tube. The meeting itself was a Specialist Discussion of the Royal Astronomical Society (RAS), on "Extragalactic Radio Sources", and began on time in the cosy lecture theatre at Savile Row. Unfortunately, however, these meetings are beginning to suffer somewhat from the problems raised by their own success. They were introduced relatively recently, in an attempt to break down some of the traditional barriers of formality at the monthly meetings of the RAS and to provide a forum for more informal discussion of specialist topics, where "A Fellow" could stand a chance of contributing something worthwhile to the proceedings.

The first series of these new meetings was a huge success, in spite of the cramped conditions at Burlington House where they were then held, and rooms were literally packed to overflowing with fellows eager to keep up with advances being made at the frontiers of astronomy. Now everyone can sit in comfort in the luxurious accommodation at Savile Row. But the speakers are removed from the audience by the usual paraphernalia of lecterns and so on, and the seats both encourage drowsiness and make Fellows reluctant to stand up and be counted. The result is that the meetings, instead of providing a lively forum, are now no more than a series of highly informed lectures, which are certainly among the best of their kind, on occasion, but which are drifting back into the rigid chairman/speaker/audience format of old.

Indeed, I confess that, presented with what seemed to be a choice of a quiet snooze or getting an early train home, I succumbed to the latter temptation and left the proceedings early. That is when my troubles began.

The nearest tube station to the lecture rooms is Oxford Circus. Progress in that direction was impeded by what seemed to be half the British student population, filling Oxford Street while demonstrating in favour of higher grants, and chanting "Heath out," a suitably apposite slogan on the day Parliament was being dissolved. But it was possible to squeeze into the station and get as far as the platform, where a loudspeaker was busily requesting passengers to leave at once because of a bomb scare. Nobody seemed too worried about this

probable hoax, except two schoolgirls who ran squealing up the escalator as if they expected to meet Donny Osmond at the top.

So it was back into the fray above, and a brisk walk down Regent Street to Piccadilly Circus, where there was no bomb scare, but the journey now involved a change at Green Park underground station in order to get to Victoria. At Green Park there was a remarkable sight—a genuine practitioner of the three card trick, actually persuading mugs to deposit money with him. The only surprising thing about the three card trick is that anyone can believe it is not fixed, but in front of an admiring audience this practitioner took two customers for a pound each, while the nearby busker, striving to earn a reasonably honest living by playing what seemed to be a bass oboe, was passed by on the other side. Perhaps there is a moral to be drawn here.

Resisting the temptation to invest £1 (it looked so easy) I hurried on to Victoria and points south. Or so I thought. A blank departure board (the biggest of its kind in the world, we are informed) told the sad story. No trains to Sussex, let alone Brighton. An appropriate end to a curious afternoon.

Push for solar power in the United States

THE US House of Representatives last week passed a bill which would provide some \$50 million over the next five years in government money to subsidise the development of solar heating and cooling devices. The bill, sponsored by Mike McCormack, a Democrat from Washington State, was passed by 248 votes to 2, a margin which indicates that in election year the energy crisis is good for voter appeal.

The idea is to add money to NASA's budget to enable the space agency to let contracts with industry for fabrication of residential heating and cooling devices. The goal is to develop 4,000 units in the five-year period. Half the units would be installed in federal buildings or federally owned houses. The other half of the units would be installed in private houses, at no cost to the owner, but they would remain the property of the US government for five years so that their performance can be evaluated.

McCormack pointed out during debate on the bill that if the programme is successful in persuading 5% of the houses and buildings in the United States to generate 80% of their heating and cooling requirements from solar energy, the bill will save 600,000 barrels of oil a year by the mid 1980s.

The bill now goes to the Senate, where prospects for its passage are bright.

Thus, with \$50 million proposed in the Administration's budget for research and development on solar energy, and McCormack's bill providing another \$50 million over the next five years to exploit the technology, those who have been advocating this energy source for years must be delighted by the advent of the energy crisis.

The only cloud that slightly dims the outlook for this bill in particular, and for the solar energy programme in general, is that there may be a fight between Congress and the Administration over who should have charge of the effort. It became clear during debate on the bill that the Administration, acting through the Office of Management and Budget, suggested to several Congressmen that NASA is not the best home for such a programme, and that the bill should be delayed until more permanent arrangements have been worked out for the management of the federal government's entire energy research and development programme. Such an arrangement—the Energy Research and Development Administration (ERDA)—has already been approved by the House of Representatives, but may become becalmed in the Senate. In the event, however, the House decided to give the authority to NASA, but to transfer the programme to ERDA if, and when, it is established. It remains to be seen whether the Administration's opposition will have any effect on the Senate's consideration of the bill, but electoral appeal is likely to outweigh White House pressure.

Exciting end to British Chess Championship

Jonathan Penrose

THE British Chess Championship was first held in 1904 and since that time, with one or two breaks, has been an annual summer event lasting about a fortnight. The result of the 1973 championship held at Eastbourne ended in a tie between Michael Basman and William Hartston (both in their mid-twenties). There was no time for an immediate play-off for first prize so that the issue hung in the balance until the week of January 28–February 3, 1974 when a six-game match was duly played in the luxurious ballroom of the Heathrow Hotel.

The styles of the two players were sharply contrasted. Hartston has a sound knowledge of theoretical lines of play (based on systematic homework of opening developments in international play) and favours a logical plan of campaign, whereas Basman is a tactician who spurns orthodoxy and seeks to create unusual positions even in the earliest stages of the game. The course

of the match (a $4\frac{1}{2}$ – $1\frac{1}{2}$ win for Hartston) tended to illustrate how hard it is becoming to be truly original in chess and at the same time maintain a basic soundness in strategy. Hartston's first British Championship win was, however, well merited.

Other matches taking place early this year are those of the quarter-finals of the 'candidates' matches. These involve a series of knock-out matches to decide who is to challenge Fischer for the World Championship scheduled for 1975. Boris Spassky (the defeated champion in 1972) seems to have regained his form, having recently won the Soviet Championship, and he convincingly defeated Robert Byrne (United States) in one of the candidates matches. His opponent in the semi-finals will be the

young Soviet player Anatoly Karpov, who is hailed as a future world champion. The match between these two should be hard-fought and close. The other half of the draw for the candidates matches also seems likely to be dominated by Soviet players with Petrosyan and Korchnoy the most likely protagonists for the semi-finals.

In the shadow of these candidates matches lies the enigma of the role that Fischer is prepared to play in them. He has not played a single serious game since his match with Spassky in 1972 and whether or not he will play the winner of the candidates matches must still be considered an open question. All chess enthusiasts must surely hope that he will play.



James Hart

SCIENTISTS at the Royal Aircraft Establishment at Farnborough agree on a show of hands to take industrial action in support of their wages claim. The Farnborough men were among the 30,000 scientific workers whom the Institution of Professional Civil Servants (IPSC) was preparing for one-day strikes and half-day walkouts in order to stir up some enthusiasm for their claim in government circles. The dispute threatened to affect 16,000 Civil Service scientists and something like 15,000 related workers in the United Kingdom Atomic Energy Authority, the Research Councils and the Veterinary Service, as well as lecturers in defence colleges, patent examiners and even the Meteorological Office's television weather men. Thousands of members took part in half-day strikes or 'non-cooperative' working at establishments throughout the country and, after a week's respite called by the

union to let the government offer an interim increase or the Pay Board an early report, the scientists warned that they could escalate their action in order to hit at 'sensitive points' relying on IPCS scientists. The dispute was caused by a disparity between the pay of scientists and that of comparable grades in the executive branch of the Civil Service. The scientists have been trying to get their pay structure re-assessed since 1971 and expected a settlement in January. Instead, they were given the news that the Pay Board was unlikely to report before March at the earliest. The General Secretary of the IPCS, Mr William McCall, says the dispute is a story of a reasonable group of workers, opposed to militancy but driven to it by bitterness and anger. It is, in his view, a case bearing the hallmarks of the exploitation by a government of the industrially weak.

correspondence

Tank mentality

SIR—It is disappointing to find the editor of *Nature* advocating the use of think tanks (*Nature*, 247, 169; 1974). A tank is conceptually a rather isolated system. It has inflow and outflow pipes and, hopefully, check valves but it does not otherwise interact with the outside world. Interaction, however, as Robert Eisler used to emphasise, is the basis of our sense of reality. It requires a tank mentality to lift the customer-contractor principle from one field, trade and manufacture, where it is appropriate, to another, research and development, where it is not. A customer may reasonably contract to purchase a specifiable product such as a type of refrigerator, the performance and production costs of which are known. In contrast, it is a grave risk (outside the tank world) to make a contract for some system which has not previously existed, in the development of which materials and systems are to be used in a new way. What but a tank mentality could give rise to phenomena like the notorious groundnuts scheme, could accept 'estimates' for the costs of certain Rolls-Royce engines or for Concorde or could envisage estimating the cost of producing a new crop variety in agriculture with specified yield and characteristics of resistance to disease.

The conflict between planning or drawing office, which produces a design, and the shop floor, which finds it impractical, is too common. As the scale of human planning increases, the drawing office becomes larger and more distant from operational reality. Planner and producer become more widely separated; more impractical, damaging or disastrous schemes are floated. What is needed is a thinker-doer mentality. Planners should leave their offices to participate in the execution of their plans to return as wiser men taught and stimulated by their shop floor or field experience.

It will be protested that some can think but cannot do and are better in the planning office, whereas others can do but cannot think and are better on the shop floor. True, but neither of these groups should be given responsibility beyond its limited experience for evaluating or developing new schemes. Positive steps are needed to provide a supply of citizens experienced and capable in both activities, thinking and doing.

Science has shown great success at

prediction from general principles but if *hubris* or just crass ignorance should lead us to underestimate the complexity of the world about us, we shall fail. Let us not forget the advice of Columella, author of that fascinating encyclopaedia of agricultural experience, the *De Re Rustica*, written in the first century of our era (I quote from the Loeb Classical Library edition, 1960, Book I, 1, 16):

"Usus et experientia dominantur in artibus, neque est ulla disciplina, in qua non peccando discatur."

"It is practice and experience that hold supremacy in the crafts, and there is no branch of learning in which one is not taught by one's own mistakes."

Yours faithfully,

H. HACK

33 Manor Rd,
Henley-on-Thames,
Oxfordshire

ERTS

SIR,—Due to postal delays I have only recently been able to read your editorial "ERTS—Technological Success, Scientific Failure?" (*Nature*, 245, 345; 1973). To me it was a thoughtful questioning of a programme involving a combination of science, technology, and bureaucracy.

I have had a bit of experience with geologic interpretation of space imagery, which has a distinct advantage when one wants to step back for a regional perspective of major structural trends that cannot at present be viewed in other ways. Mosaics of aerial photographs are almost always uneven in quality, the unevenness greatly inhibiting a regional analysis. Enough has been said about the necessity of ground-truth (confirming one's interpretation of imagery data by checking features on the ground) for one to appreciate the work and expense involved in analysing ERTS imagery. It is worth emphasising (as you did) that the resolution is only sufficient for viewing large features, although, as regional geologic trends can often be viewed as large features ERTS is still quite useful here. In other words, aerial and space imagery are not mutually competitive, but are complementary.

You also rightly note that weather and constant sun angle conditions mask many areas as well as many features within a given area. But weather conditions are as much a problem for conventional aerial surveys in areas such as tropical, developing Ghana. Here a

Canadian team has been stationed for a couple of years trying to conduct a survey that is usually hampered by cloud cover during the rainy season, and thick dust cover for much of the dry season. Nevertheless, the crew must stay here to be prepared to fly on that rare day when conditions are right. Similar climatic hindrances exist over much of the earth's surface; and I am sure that quite a lot of money is similarly spent on conventional aerial surveys elsewhere with only meagre results during long periods, unless one is satisfied with only radar imagery (which is better than nothing, but has considerable limitations). ERTS may not be especially expensive in this regard at least in operating costs, when scale of coverage is considered.

As an earth scientist I feel that ERTS must be tested for some time, eventually proving itself a scientific, technological, and economic success or failure. What worries me is that the agencies involved, such as NASA and the United States Geological Survey's Earth Resources Observation Systems (EROS) programme might, for short term prestige purposes prematurely push to prove ERTS by spending on expansion before such expansion is justified. There certainly are indications of overeagerness to declare ERTS a success, and to rapidly expand the programme. For example, about three years ago, when EROS was pushing for—and receiving—large budget increases, I saw a promotional brochure proclaiming that "automated snow mapping" had been accomplished. Having done field work in their study area I had a good chilly laugh noting that their claimed delineation between "thick" and "thin" snow cover was actually a map of the tree line! In their haste to 'prove' a point, they must have failed to make that essential ground-truth check.

Though I disagree with you in some details, my general opinion is similar. NASA shouldn't consider ERTS-B until scientists and technologists outside the sponsoring agencies have concluded that a successor to ERTS-A would be worthwhile, and that at least part of the additional sea of data would be analysed. But ERTS-B should not be forgotten, only relegated to the subconscious.

Yours faithfully,

DAVID A. HASTINGS

Department of Physics
University of Science and Technology
Kumasi, Ghana

news and views

Homology and structure of transfer RNA

MUCH interest at present centres on initiator transfer RNA which in its aminoacylated form donates a methionine residue to the N-terminal position of nascent proteins in the initiation of protein synthesis. These tRNA^{Met} species are distinguished from other methionine specific tRNAs in that they can only participate in initiation and not in elongation; this second role is reserved for tRNA^{Met}. All the initiator species have several common biochemical properties in that they can all act as substrates for *Escherichia coli* methionyl-tRNA synthetase and for *E. coli* methionyl-tRNA transformylase. On the other hand initiators from prokaryotes and cell organelles are distinguished from those from eukaryotic cytoplasm in that the latter are not formylated *in vivo*. There is also some evidence that the course of initiation in mammalian cytoplasm proceeds in a different manner than in prokaryotes; thus the binding of tRNA^{Met} to the 40S ribosomal subunit in mammals is independent of template suggesting perhaps that the tRNA bound to the ribosome selects the initiation region of the mRNA rather than the reverse.

It is to be expected that these differences and similarities in the behaviour of initiator tRNA species is reflected in their three-dimensional structures and hence in their sequences. Research by two groups of workers reported on pages 516 and 518 of this issue of *Nature* suggest that this is the case.

Piper and Clark describe the complete sequence of cytoplasmic tRNA^{Met} from mouse P3 myeloma cells and Simsek, RajBhandary, Boissard and Petrisant give that for rabbit liver cytoplasmic initiator tRNA. It turns out that these two sequences are identical. Moreover, Simsek *et al.* provide strong evidence based on the identity of oligonucleotide fingerprints that the initiator from sheep mammary gland cell cytoplasm is also the same. It would thus seem that all mammalian initiators are identical. Although this identity does not extend to all initiator molecules, comparison with other published sequences shows extensive homologies between the mammalian sequence and that from yeast; indeed, notable homologies even exist with *E. coli* tRNA^{Met}. No doubt these homologies are related to the biochemical similarities between the initiators already noted, as well as their special role in protein synthesis.

On the other hand, the prokaryotic and eukaryotic initiators are sharply distinguished in that the latter—in mammals and yeast—both possess in loop IV of the clover leaf structure the unique sequence A-U-C-G-m¹A-A-A whereas *E. coli* has the sequence T-Ψ-C-A-A-U with the conventional T-Ψ-C triplet and a pyrimidine in the last position. Unpublished work quoted by Simsek *et al.* shows that plant cytoplasm initiator also has A-U*-C-G-m¹A-A sequence with a modified U so that the odd sequence seems to be universal in eukaryotic cytoplasmic initiators.

Another feature of interest in the mammalian sequence is the presence of a C residue immediately preceding the anticodon instead of U which is otherwise universal. This feature is absent in yeast and its significance remains unclear.

Piper and Clark quote unpublished work to the effect that the two isoaccepting tRNA^{Met} species from mouse myeloma cytoplasm contain conventional sequences in loop IV. These

molecules are not formylated but they do act as substrates for *E. coli* methionyl-tRNA synthetase. These observations strengthen the notion that the unconventional sequence in the eukaryotic initiators is related to their peculiar role in protein synthesis. It is to be presumed that this relation is mediated through alterations in the three-dimensional structure of loop IV but at present there is no evidence on this point.

The question of the secondary structure of tRNA has been taken up by Kearns, Wong, Hawkins and Chang in a communication in the same issue of *Nature* (page 541). These authors are concerned with the difference between the secondary structures of native and denatured tRNA as manifested particularly by yeast tRNA^{Leu} and they have compared the high resolution nuclear magnetic resonance (NMR) signals obtained from these two stable conformers. Previous work had established that each Watson-Crick base pair produces a single resonance in the low field (11–15 p.p.m.) region and analysis of the low field spectrum of the denatured and native forms of tRNA^{Leu} revealed that the first has 18 ± 2 base pairs and the second about 4 more. The detailed analysis

Oil and Earthquakes

A REMARKABLE and exciting set of observations is reported by Arieh and Merzer on page 534 of this issue of *Nature*. Oil-field technology and earthquake prediction are brought to each other's aid by means of an astute comparison.

For several years the oilflow from wells in the Gulf of Suez has been subject to periods of large fluctuations interspersed between times of relative uniformity. These periods have correlated across a set of several wells. Oil is forced out of the wells by the hydrostatic pressure of the overlying rocks and so fluctuations in flow are presumably caused by variations in the pressure at depth. The weight of the rocks clearly does not change so what are observed must be variations in the way that this pressure is transmitted to the interstitial fluid. That is, effects of varying pore pressure are being seen. Arieh and Merzer observe that the times of maximum fluctuation precede the occasions of large earthquakes a hundred kilometres away at the north end of the Red Sea. The quakes seem to restore calm to the oil flow. Their first figure tells the whole story.

The observations cannot but strengthen the seismologist's belief that some earthquakes are predictable by precursory dilatancy changes in the rocks in the vicinity of the focal region. The time duration of the effect is in good agreement with previous approximate relationships between precursor time and magnitude. Moreover, the observations demonstrate that the dilated region is large, also in agreement with other work that has been published during the past year.

Oil companies and earthquake geophysicists have a lot to tell each other in the months ahead.

D.D.

of the spectra also showed that the difference between the two forms is not merely that the D form has lost a few base pairs. Instead, so the data imply, at least five pairs are lost from the N structure and two reformed in the denaturation reaction. Thus the secondary structures of the two forms are different and not merely variants produced by a small unfolding of the clover leaf structure. Semi-empirical rules already available allow the calculation of the NMR spectrum corresponding to a given secondary structure and there is good agreement between the observed spectrum of the N form and that calculated for the conventional clover leaf model.

Kearns *et al.* investigated several other alternative structures and compared their calculated spectra with that observed for the D form. All the models tried retained the amino acid acceptor stem for several reasons including the observation that the D form competitively inhibits the aminoacylation of the N form. The model which gave the closest fit between theory and experiment incorporates the feature that the DHU and the anticodon stem are replaced by a helix of five base pairs formed between residues in the T- Ψ -C loop and in the anticodon region. Conversion of the D to the N form would require the disruption of these five base pairs and could account for the high activation energy for this process. The validity of this model is strengthened by the fact that it is consistent with the oligonucleotide binding data reported by Uhlenbeck *et al.* (*Fed. Proc.*, **31**; 1972).

From a Correspondent

Nuclear and messenger RNA

THERE has long existed a prejudice that in higher animals, and in particular in birds and mammals, messenger RNA is first synthesised as a very much longer precursor RNA molecule, usually called heterogeneous nuclear RNA (HnRNA) because of its large and variable size. This is broken down in the nucleus to give mRNA (to be exported to the cytoplasm) and degraded fragments. Harris (*Biochem. J.*, **84**, 60; 1962) noted more than ten years ago that most of the RNA synthesised in the nucleus is broken down there and never exported to the cytoplasm. The nuclear RNA, however, is for the most part very large, and in sucrose gradients sediments at up to 70S (molecular weight approximately ten million); therefore, most of it might be degraded and yet the remainder yield molecules of the size of cytoplasmic messenger RNA (molecular weight about 250,000).

The original investigations of the relations between nuclear and cytoplasmic RNAs were carried out, in particular, by Darnell, Georgiev and Scherrer. Darnell has recently reviewed both the history and the evidence of the existence of precursor mRNA in the nucleus in a very lucid article (*Science*, **181**, 1215; 1973).

Direct evidence for the existence of mRNA in a large molecule in the cell nucleus can only come by a definitive identification of a specific messenger RNA, either by its translation into a protein or its sequence identification by DNA:DNA hybridisation or fingerprinting. The fact that a portion of the high molecular weight RNA of the nucleus contains poly (A) at its 3' end certainly suggests a relation between it and cytoplasmic messenger RNA (all of which contain poly(A) sequences apart from histone mRNAs), but is not a proof positive. It is particularly important to show that RNA from the cell cytoplasm has not aggregated in a non-specific way with other nuclear RNA to give large mRNA-containing material which is not a primary transcript.

Three groups have now published data directly translating specific proteins from mRNA. Stevens and A. Williamson (*Nature new Biol.*, **245**, 101; 1973) have made use of the

very tight binding found between heavy chain immunoglobulin mRNA and the complete myeloma protein (H_2L_2). In this way they purified the nuclear precursor RNA for the heavy chain mRNA and shown it to have a molecular weight of two million or greater. The nuclear pre-messenger causes immunoglobulin synthesis in *Xenopus* oocytes when injected.

In a similar series of experiments, R. Williamson and his colleagues (*Nature new Biol.*, **241**, 66; 1973) have micro-injected HnRNA from mouse foetal liver cells into oocytes and obtained mouse globin synthesis. Since the RNA was purified using a zonal rotor, non-specific contamination is unlikely. Brain HnRNA prepared in the presence of reticulocyte polysomes synthesising mouse globin did not cause globin synthesis in oocytes.

Neither of these sets of experiments completely rules out the possibility of a specific interaction between a small nuclear mRNA and a larger nuclear RNA molecule which, for instance, transports it into the cytoplasm. In order to prove that such interactions are not causing the apparent large size of the nuclear RNA, it is necessary to show that these are still as large even when treated with solvents which break non-covalent interactions. Such hydrogen bond-breaking solvents include dimethylsulphoxide and formamide.

Ruiz-Carrillo and his colleagues (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 3641; 1973) recently published a conclusive demonstration that large nuclear RNA from duck reticulocytes (unlike mammalian reticulocytes, those from birds have nuclei) contains globin mRNA sequences, even after centrifugation through a gradient containing 99% dimethylsulphoxide. Such a solvent would certainly denature all hydrogen-bonded aggregates, whether arising non-specifically during RNA isolation or during the synthesis of the RNA. Even nuclear RNA sequences larger than 45S code quite efficiently for duck globin in a cell-free protein synthesis system derived from mouse Krebs tissue culture cells. When rabbit globin mRNA, which sediments at 9S (molecular weight of 220,000), is added to the RNA before centrifugation, it does not contaminate the large nuclear RNA, as shown by the absence of rabbit globin synthesis.

As is well known to readers of these columns, an enzyme from RNA tumour viruses, reverse transcriptase, can make an exact complementary DNA copy (cDNA) from a messenger RNA template using an oligo(T) primer. Such a complementary DNA, which can be synthesised at a high level of radioactivity (up to approximately 80 million counts per min per microgram), can then in turn be used as a probe to search for messenger RNA or messenger gene sequences, even if these are present in very low amounts. The first attempts to utilise such techniques used not cDNA made with reverse transcriptase, but what might be called cRNA prepared with RNA polymerase (Melli and Pemberton, *Nature new Biol.*, **236**, 172; 1972). These results demonstrated the existence of globin mRNA sequences in duck nuclear RNA, but in this case disaggregating conditions were not used.

Scherrer and his colleagues (*Proc. natn. Acad. Sci., U.S.A.*, **70**, 1122; 1973) used cDNA prepared from duck globin mRNA and annealed it to different size classes of large duck nuclear RNA. They showed that small amounts of globin mRNA sequences could be found even in RNA of molecular weight approximately ten million, although only perhaps 1% of the molecules of this size contained a globin mRNA sequence. The large nuclear RNA had to be denatured before the interaction could take place, indicating that it might be bound to other RNA sequences intramolecularly.

Recently two studies of the physical behaviour of giant nuclear RNA have appeared, one using material from sea urchins (Peltz, *Biochim. biophys. Acta*, **308**, 148; 1973) and the other from rat ascites cells (Holmes and Bonner, *Biochemistry*, **12**, 2330; 1973). In both cases very convincing evidence is presented that the RNA is still very large after

denaturation, and is not an aggregate of messenger-sized molecules. Such evidence must encourage those who have argued for a long primary gene transcript, much longer than the ultimate cytoplasmic messenger.

Many questions are, however, still unanswered—Darnell outlines some in his paper in *Science*. I shall focus on just two. What is the significance of the large amount of secondary structure found in nuclear RNA? Might it represent a signal which determines the sites of cleavage before processing to the cytoplasm, perhaps ancillary to the poly(A) sequence and related to it? (It is known that short oligo(U) sequences do exist in large nuclear RNA, and might, of course, be signals which interact with the poly(A) at the 3' end. It is surely interesting that the enzyme which processes RNA in *Escherichia coli* is a double-strand-specific RNase II (Dunn and Studier, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3296; 1973).)

The second question is the one which has always been intriguing—why is so much RNA synthesised anyway? It is known that *in vivo* as much as 10 or 15% of the DNA of the cell is transcribed, yet only a tiny fraction of this has a known function. Now it can be said with some certainty that each mRNA molecule contains, when made, a tail some ten or twenty times larger than itself, again of unknown usefulness. Moreover, only a proportion of the nuclear HnRNAs, even in a differentiated cell, contain poly(A). All of the recent attention has focussed on proving the existence of the messenger portion of the HnRNA. In future it may be the other sequences, among which controlling elements might be found, that will command more interest.

R.W.

Formation of the Himalayas

from our Geomagnetism Correspondent

IN their landmark paper on plate tectonic mountain building, Dewey and Bird (*J. geophys. Res.*, **75**, 2625; 1970) took the formation of the Himalayas to be a typical example of continent-continent collision, notwithstanding the fact that many workers have pointed to differences between the tectonic histories of the Himalayas and the Alps. But as Powell and Conaghan (*Earth Planet. Sci. Lett.*, **20**, 1; 1973) have now pointed out, although the Himalayas are obviously not cordilleran-type mountains, some of the criteria set up by Dewey and Bird and by Dewey (*Earth Planet. Sci. Lett.*, **6**, 189; 1969) to distinguish between collision and cordilleran belts are apparently not applicable in this case. For example, in cordilleran mountains gravity slides pre-

date hard rock thrusting whereas in collision mountains the opposite occurs; the dominant metamorphic assemblages in collision mountains are in the blueschist facies in contrast to the paired metamorphic belts of cordilleran mountains; and whereas subduction continues during the formation of cordilleran mountains, it is terminated in collision mountain building by the buoyancy constraints on substantial underthrusting of continental crust. The strict application of these criteria would thus suggest that the Himalayas were not formed as a result of continent-continent collision—a conclusion which would clearly be wrong.

So how can this paradox be resolved? The answer, according to Powell and Conaghan, is that although the Himalayan mountain chain is basically of the collision type it was not formed as a direct result of the collision but as a result of uplift during underthrusting along a deep crustal fracture. The development of the Himalayas apparently took place in two stages. During the first of these the northward-drifting Indian subcontinent converged on and ultimately collided with a proto-Tibetan landmass. The second stage then began with the formation of a fundamental crustal fracture within the Indian block and continued with the underthrusting of the Indian subcontinent along this fracture.

In greater detail, the model proposed by Powell and Conaghan, and based on an examination of the structural and stratigraphic record, begins with the situation before 130 million years ago. At this stage the Indian block was drifting northwards towards the Asian continental block, the two land masses being separated by the shrinking Tethys

Ocean. The stratigraphic record in the Spiti area of what is now northern India and southern Tibet indicates that open marine sedimentation was taking place on the continental terrace at the north of the Indian block during the Triassic and Jurassic, the deposits being predominantly carbonate and fine-grained terrigenous rocks. Fossils at the base of the Giumal Sandstone which overlies these deposits are no older than 130 m.y. and fossils in the higher Chikim Series are no younger than 65 m.y., showing that the Indian and Asian blocks were still converging between these dates. The stratigraphic record in the vicinity of the present Indus suture indicates that throughout this period subduction was taking place at the southern edge of the Asian block—that is, subduction of the northward-drifting lithosphere carrying India.

The collision of India and Asia along what is now the Indus-Brahmaputra suture occurred before the middle Eocene (about 45 m.y.), but it seems unlikely that any mountains were thus produced. According to the Powell-Conaghan model, the first result of this original collision was a down-buckling at the margins of the Indian and Asian blocks as the subducting Indian plate, constrained by the buoyancy of its continental crust, became jammed. This led directly to the middle Eocene marine transgression of northern India, but by this time neither the central gneisses and schists of the High Himalaya nor the southward thrusts of the Lesser Himalaya had formed.

The period between the middle Eocene and the early Miocene (about 20 m.y. ago) was marked less by relative movement between India and Asia than by post-collision isostatic adjustment lead-



An Everest panorama (copyright: *The Times*).

ing to the Oligocene regression. It was during this period also that the gneisses and schists of the High Himalaya were produced, although the precise mechanism of formation has not yet been worked out. In any event, the narrow gneiss-schist zone formed some way to the south of the northern margin of the original Indian block, effectively splitting off the northernmost 100 km or so of the Indian crust. By the early Miocene the reduced frictional resistance in the gneiss-schist zone enabled the southern segment of the Indian lithospheric block to begin underthrusting; and by 5 million years ago it had underthrust both the original northern segment of the Indian crust and part of the Asian crust (not lithosphere). Where underthrusting had occurred there was, of course, a double layer of crust; and the resulting gradual isostatic uplift added to the upthrust of the Himalayas which had commenced as soon as the underthrusting began, as well as producing the Tibetan plateau. In fact, the Himalayas did not reach their present elevation until late Pliocene or Pleistocene, which fact led Gansser (*Geology of the Himalayas*, Wiley-Interscience, 1964) to speculate that prehistoric man may have witnessed their rise.

This is, of course, just an outline of the processes described in greater detail by Powell and Conaghan, who go on to give supporting evidence from seafloor spreading rates and from the sedimentation patterns in the deep sea fans associated with the Indus and Ganges/Brahmaputra river systems. But the central point is clear—the Himalayas were produced not directly by the continent-continent collision but indirectly by the subsequent underthrusting. As a result, since the early Miocene the active tectonic zone has been not the original site of continental collision marked by the Indus-Brahmaputra suture but the central crystalline axis of the gneiss-schist zone marking the High Himalaya (where current seismic activity is greatest). A more detailed consideration of the processes involved also explains why the Himalayan chain fails to conform with the three Dewey-Bird criteria for recognising single-stage collision-type mountain building.

Transitions in the sarcoplasmic reticulum

from a Correspondent

MEASUREMENT of the effects of temperature on transport processes in several microbial systems has led to correlations between mobility of lipids and transport activity. More detailed experiments with purified transport systems are beginning to appear. Even with these simplified systems the results are interpretable

only in the most general terms, as shown by a thorough examination of the Ca^{2+} transporting vesicles from sarcoplasmic reticulum (Inesi, Millman and Eletr, *J. molec. Biol.*, **81**, 483; 1973). These vesicles contain few proteins and the major one, a Ca^{2+} activated ATPase which accounts for 70% of the membrane protein, is by itself able to transport Ca^{2+} across the membrane. The lipids are highly unsaturated and little cholesterol is present.

In earlier papers Inesi *et al.* reported changes in the slope of Arrhenius plots of the motion of several different nitroxide spin labels either linked to the —SH groups of the ATPase or incorporated into the lipid bilayer. The spin-labelled fatty acids in the lipid bilayer gave a change in slope at about 20°C which was not affected by prior heat denaturation of the protein. The maleimide spin labels coupled to the ATPase protein also showed a slightly increased slope below 20°C, and a more marked one above 40°C. The low temperature change seems to be a function mainly of the lipid phase since it can be correlated approximately with a change in rate of Ca^{2+} transport by a lipid soluble ionophore (Scarpa *et al.*, *J. gen. Physiol.*, **60**, 735; 1972). The nature of the change is obscure since the main order-disorder transition in systems of highly unsaturated lipids is below -20°C, but a parallel may be provided by changes in physical and transport properties of other unsaturated lipid systems in the neighbourhood of +20°C (for example, Esfahani *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **68**, 3180; 1971).

In their latest paper, Inesi *et al.* have correlated these two transitions with changes in ATPase activity and in the rate of Ca^{2+} transport. When the temperature was increased above 20°C, the heat of activation of both processes decreased from the very high value of 27 kcal mol⁻¹ to 17 kcal mol⁻¹. It therefore seems that changes in the lipid environment are reflected in changes in activity of the protein. Above 40°C, net Ca^{2+} uptake ceased but ATPase activity continued unchanged; other evidence suggested that a change in the protein was involved. It is of interest that in the course of their recent determination of the diffusion constants of phospholipids in sarcoplasmic reticulum membranes, Scandella, Devaux and McConnell, *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2056; 1972) found that this loss of Ca^{2+} transport at 40°C was prevented by 30% sucrose. This is but one of many examples of the sensitivity of this system to changes in the environment.

Although these transitions undoubtedly reflect interesting happenings within the sarcoplasmic reticulum, it will not be possible to interpret them in molecular terms until one has a better under-

standing of the structure of the ATPase protein and can incorporate it in a fully functional state into well sealed vesicles of purified lipids.

Quick measurement of carrier mobility

from our Solid State Physics Correspondent

AMONG experimental solid state physicists, the interpretation and measurement of carrier mobility in semiconductors or insulators has an aura of difficulty. Not only is the path of an electron, drifting in a field within a solid, influenced by many different forms of scattering (for example interaction with phonons, impurities and vacancies in a variety of charge states) but the measurement is not simple. For the usual Hall method, precision-cut samples with at least four low resistance ohmic contacts are needed and the resulting 'Hall mobility' has to be corrected in order to find actual drift mobility. This characteristic of carriers must, however, still very frequently be determined during research on semiconductors and so much time and patience are expended by physicists in getting valid mobility data. That is why, when a 'quick' method for the measurement of mobility is announced, interest is naturally aroused.

Howes (*Rev. Scient. Instrum.*, **44**, 1223; 1973) has described an evaluation of a 'direct-reading' method, using measurement of magneto-resistance by a nulling technique, involving two terminals rather than four. The latter feature brings advantages over and above the obvious one that preparation time is reduced. Many gallium arsenide transferred-electron devices, made for microwave generation studies, already have the right geometry of semiconductor chip and thus two correlated measurements can be performed on the same sample. This is particularly important when, as is often the case, different samples, even if cut from the same semiconductor ingot, may exhibit different impurity concentrations and carrier mobility values.

The new method relies on the fact that, for a sample of low aspect ratio (distance between terminals small compared with other dimensions), the applications of a magnetic field produces a reduced current flow. This results from the fact that, unlike the case of 'Hall bars' (which have a high aspect ratio) the transverse 'Hall Field' is not sufficient to overcome the Lorentz force which always reduces the near free path of carriers in the direction of the current flow. The decrease of this mean free path is reflected in the current passed for a given voltage and, in fact, a simple

expression can be derived in which the absolute value of mobility is proportional to the square root of the sample resistance change (as a fraction of the original resistance and using unit magnetic field). It is thus a simple matter to balance a resistance bridge with the sample in one arm at zero magnetic field, then apply the magnetic field and use electronic means to rebalance the bridge. The electronics can then indicate the resistance change directly or, by using a circuit which performs a square-root operation, indicate directly the absolute value of 'magnetoresistance mobility'.

Howes has compared Hall mobilities with the magnetoresistance mobilities for

several samples of germanium, silicon, gallium arsenide and indium phosphide and found that the ratio of the two values rarely varies more than 5% from unity. Thus, the method can at worst be described as a rough-and-ready Hall measurement and, at best, could make some tedious forms of Hall measurement unnecessary.

Integration of proviruses

from a Correspondent

It is now 10 years since Temin postulated that RNA tumour viruses replicate by way of a DNA intermediate, the provirus. This hypothesis, for many years regarded as heresy, gained general acceptance in 1970 with the discovery of an RNA-dependent DNA polymerase (reverse transcriptase) in RNA tumour virus particles. Last year, Linial and Mason (*Virology*, **53**, 258; 1973) reported that the reverse transcriptase does indeed play an essential part in the function of these viruses because mutants of Rous sarcoma virus, which are temperature sensitive for the enzymatic activity, cannot replicate in nor transform cells infected at the non-permissive temperature.

It has proved more difficult, however, to demonstrate the formation of proviruses inside infected cells. Until recently, attempts to find new proviruses by molecular hybridisation have been thwarted by the presence of endogenous viral genetic information in the natural host cells. Schincariol and Joklik (*Virology*, **56**, 532; 1973) have now demonstrated the formation in infected cells of additional sequences of DNA homologous to the RNA of Rous sarcoma virus (RSV). Schincariol and Joklik used a sensitive single-stranded DNA probe synthesised from RSV RNA by reverse transcriptase in the presence of actinomycin D. The DNA represents more than 90% of the RNA genome. Reassociation kinetics of the complementary DNA in the presence of excess DNA prepared from infected and uninfected chick cells indicate that two copies of RSV provirus are made in addition to the one viral genome equivalent endogenous in chick cells. By hybridising excess RNA prepared from cells at different times after infection to the single-stranded DNA probe, Schincariol and Joklik also examined the initiation of transcription of the proviruses and found that 100 copies were transcribed within 24 h of infection.

The complicating factor of endogenous viral genetic information may be avoided by infecting unnatural host cells; for instance, mammalian cells do not have DNA homologous to avian tumour virus RNA. Thus Varmus,

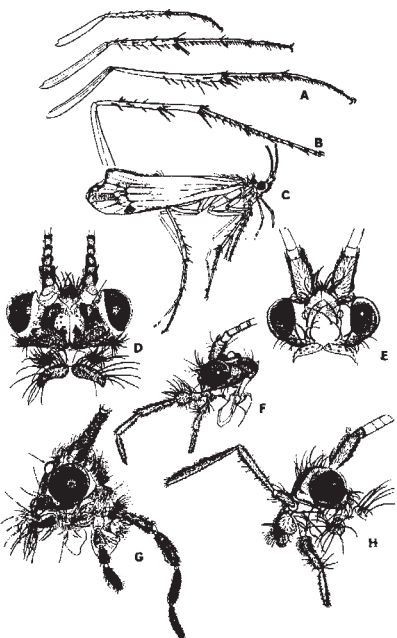
Vogt and Bishop (*J. molec. Biol.*, **74**, 613; 1973) found two to four avian proviruses in mammalian cells transformed by RSV but none in uninfected cells. Varmus, Vogt and Bishop (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 3067; 1973) now report that the viral DNA becomes covalently linked to host DNA following transformation of mouse, rat, or duck cells with RSV. Varmus *et al.* make use of Britten's observations that most high molecular weight DNA extracted from higher organisms contains reiterated sequences. When unshredded DNA is incubated to low *C_{ot}* values at which repeated, but not unique, sequences reassociate, 'networks' of DNA are formed which can be separated from the remainder of the DNA by sedimentation. Much of the DNA homologous to viral RNA in transformed cells was found in the networks, thus demonstrating its integration into the host genome.

Varmus *et al.* then examined the formation and integration of viral DNA in freshly infected cells. Duck cells are permissive for replication of certain strains of RSV and were found to contain no endogenous sequences homologous to the RNA of RSV. Virus-specific DNA was detected within 3 h of infection and using the 'network' assay, integration of viral DNA was detected from 6 h onwards. Mouse 3T3 cells are not permissive for RSV replication and have a very low efficiency of transformation by RSV, relative to chick or duck cells. Readily detectable levels of RSV DNA, however, were found in 3T3 cells within 12 h of infection, though the RSV DNA did not become integrated until several hours later. These observations suggest that the RSV particles enter and form proviruses in 3T3 cells at an efficiency comparable to that in permissive cells, although transformation is less than one thousand times as efficient. It will be interesting to see whether viral RNA is transcribed off the proviruses in these 'silent' infections.

Integration of the DNA provirus provides an explanation how RNA tumour viruses, like DNA tumour viruses, can become preserved in infected cells and cause stable transformation. Does this mean that all integrating viruses could be tumour viruses? One exception is Visna virus, which causes a 'slow' demyelinating disease in sheep. It has not been associated with any neoplastic disease although Takemoto has observed cell transformation in culture by Visna. Visna is an RNA virus that resembles RNA tumour viruses in structure and carries a reverse transcriptase. Haase and Varmus (*Nature new Biol.*, **245**, 237; 1973) have investigated the replication of Visna virus during lytic infection off sheep choroid plexus cells.

Key to caddis

LIMNOLOGISTS who have been brought up with the Scientific Publications of the Freshwater Biological Association will welcome the latest addition to the series—a key to the adults of the British Trichoptera by T. T. Macan (No. 28, £1.25). Caddis flies are not the easiest of freshwater animals to identify and this key, illustrated with drawings of specimens which have not been allowed to shrivel, should greatly simplify study of the order. In this figure, one of many drawn for the key by C. Joan Worthington, (a) legs of *Mesophylax impunctatus*; (b) mid-leg of *Agrypnia pagetana*; (c) *Glyptotaelius pellucidus*, preserved specimen; (d-h) heads from above and from side; (d) *Phryganea varia*; (e) *Limnephilus rhombicus*; (f) *Rhyacophila dorsalis*; (g) *Phryganea varia*; (h) *L. rhombicus*.



Using the 'network' hybridisation method, they show that it forms a DNA provirus which becomes integrated into the host genome.

Vanishing interstitial in semiconductors

from a Correspondent

RECENT work by a group of American workers (Weigel *et al.*, *Phys. Rev. B*, 8, 2906; 1973) helps to clear up one of the current problems in solid state physics—the mystery of the vanishing interstitial in semiconductors.

When a crystal is irradiated with high energy electrons or other particles, atoms are knocked off their lattice sites. The simplest defects thereby created are the Frenkel pair—the vacant lattice site (the vacancy) and the ejected atom (the interstitial, so called because it occupies one of the interstices of the lattice rather than a normal substitutional site). Of the elemental semiconductors (diamond, germanium and silicon) the latter has received most attention experimentally and vacancies have been detected, both alone and in combination with other defects. The problem is that nobody has yet observed the interstitial, in spite of many attempts to do so. It seems that this defect is very mobile and can diffuse over long distances until it recombines with a vacancy or is trapped by a dislocation or reaches the surface of the crystal, thereby disappearing. But as everybody is aware these days, the ability to travel a long way depends on the availability of a supply of energy. In the normal atomic transport mechanism the energy is thermal; the transistors are made by diffusing in impurities at temperatures in the region of 1,000° C. But the silicon interstitial seems to be mobile at 4 K, making it unlikely to be a thermal process.

The calculations of Weigel *et al.* are for carbon (diamond) because the approximation used, Extended Hückel Theory, has been very successfully applied to organic molecules; but the results are expected to apply to silicon and germanium as well. The solution of the mystery has two aspects. First, one would expect that an interstitial atom would occupy the largest interstice available. In the silicon lattice this is the tetrahedral site, equidistant from four lattice atoms. But the calculations show that this is not the case. The interstitial combines with a lattice atom, pushing it slightly out of position. Neither of the atoms occupies the lattice site but are both symmetrically displaced from it along the (100) crystal direction (the 'split-(100)' interstitial), rather like two people trying to sit on

one chair. This situation is of lower energy than one lattice atom plus an interstitial and hence is more stable. It also explains why previous calculations, which assumed a tetrahedral site, were not very successful.

The second aspect of the solution is that there is not one stable situation, but two, the other being the 'bond-centred' configuration, where the interstitial sits midway between two nearest neighbour lattice atoms which are symmetrically displaced from their sites, like three people sitting on a two-seater settee. Furthermore, which of these two 'interstitiality' configurations has the lower energy depends on the charge state and this can change during irradiation due to the tremendous amount of ionisation created. Consequently the interstitial would switch from one configuration to the other and hence migrate through the lattice whatever the temperature. This mechanism, 'ionisation-enhanced diffusion', was suggested earlier by Bourgoin and Corbett (*Phys. Lett.*, 38A, 135; 1972).

The authors are properly cautious about their results. For example, Extended Hückel Theory is of necessity a rather simple approximation because of the complicated nature of the calculations. And strictly speaking the extension to different charge states is not valid. It seems likely, however, that a more rigorous calculation would reach the same conclusions.

Nature of scrapie agent

from a Correspondent

WORK on the agent of scrapie, one of the spongiform encephalopathies, continues to be both intriguing and frustrating. At a meeting of the British Photobiology Society on January 16 at Imperial College, London the latest attempts to characterise the active macromolecules of this agent were discussed. Two introductory lectures by I. A. Pattison and D. A. Haig (both of the ARC Institute for Research on Animal Diseases, Compton) outlined the pathogenesis of scrapie and in particular how it differs from conventional virus diseases. Haig and his colleagues have found that the scrapie agent (SA) multiplies in cultured mouse brain cells but with one remarkable difference from normal viral proliferation—there is a strict correlation between the number of cells and SA activity. As the cells divide, the infectivity increases on a one for one basis. Approximately 100 cells are required to cause infection in a test animal.

G. D. Hunter, also from the Institute for Research on Animal Diseases, favours a structure for the SA which involves a small piece of RNA closely

associated with plasma membrane. His conviction is based on his own work, which has shown conclusively that the plasma membranes are the sites of the SA, and on work by T. O. Diener in the United States on the very small host-dependent potato RNA viroids which exhibit some of the physico-chemical properties of the SA. Some caution must be observed, however, when comparing the properties of the RNA potato viroids, which are extracted in a relatively pure form by classical nucleic acid techniques, with those of the SA which has proved difficult to isolate by such methods.

T. Alper (MRC Experimental Radiopathology Unit, Hammersmith Hospital) who, in collaboration with Haig, was responsible for early work in determining the size of the infective particle by the use of ionising radiation, reported that like some biologically important membrane systems the sensitivity of the SA to ionising radiation is much enhanced by the presence of oxygen when irradiated in aqueous suspension, whereas biologically active nucleic acids and proteins are typically no more sensitive.

Finally, R. Latarjet (Fondation Curie, Paris) suggested four possible structures for the SA which could explain why it is highly resistant to ultraviolet light. These were: a very small piece of nucleic acid; a larger piece of nucleic acid which suffers ultraviolet damage but is repaired in the host with high efficiency; nucleic acid protected by other constituents of a complex; and, lastly, a non-nucleic acid agent. Results of Latarjet's work in collaboration with Alper, Haig and Clarke, who attempted to characterise the SA by studying its response to various wavelengths of ultraviolet light, suggest that the SA may be non-nucleic acid in structure. Latarjet, however, could not at present rule out a combination of nucleic acid and glycoprotein where energy absorbed in the glycoprotein at wavelengths not characteristic for nucleic acids is transferred to the nucleic acid moiety.

Absorption of toxins by leaf surfaces

from our Plant Ecology Correspondent

It is well known that vegetation acts as a filter for particulate and aerosol material carried in the atmosphere. The effects of both impaction upon solid surfaces and settlement in situations of low wind speeds lead to the accumulation of material on the surfaces of plant leaves and stems. Suspended matter in the atmosphere may originate from the oceans, from wind eroded soils, or from industrial activities; some matter can be re-

garded as beneficial in that it is composed of substances of nutritional value to plants, whereas other materials are undoubtedly toxic. The arrival of such airborne matter in an ecosystem represents an input of nutrient or pollutant and its presence raises several questions of ecological interest. Is the nutrient or toxin input of significant importance in relation to the quantity and rate of movement of that substance in the system already? Is the vegetation which acts as a filter able to absorb the material through the leaves? Is the material washed from the canopy to the soil surface and, if so, is it taken up by plant roots? Of what importance is the material (particularly if it is a pollutant) to consumer organisms especially at the higher levels of food chains?

Goodman and Roberts (*Nature*, **231**, 287; 1971) found that considerable quantities of toxic metals can arrive at a vegetation surface as dust. Little (*Environ. Pollut.*, **5**, 173; 1973) has now raised further questions regarding the behaviour of metal contaminants within ecosystems. He attempted to ascertain whether zinc, lead and cadmium are present chiefly on the surfaces of leaves and can be removed by washing, or whether they are absorbed into leaf tissue. He also examined three different tree species in the Bristol area—*Ulmus glabra* (elm) *Quercus robur* (oak) and *Salix alba* (willow)—to find out whether they respond differently with respect to the three metals.

Little's report provides some useful information on the behaviour of toxins on leaf surfaces. Washing elm leaves in distilled water removed 67% of their total zinc burden, 87% of their lead and 62% of their cadmium. It seems therefore that the bulk of these metals is present as a deposit on the leaf surfaces and can be carried to the soil by rain-washing. Most of the remainder could only be removed by washing with dilute nitric acid, which implies that this fraction is bound more securely to the leaf surface, possibly by exchange phenomena. Boiling in deionised water for 1½ h removed the portions of the metals present in the cuticle and within cells; they comprised 7% of the total zinc, 2% lead and 4% of the cadmium in the elm leaves. This tendency for a greater degree of incorporation of zinc and cadmium into the leaf structure reflects the higher solubilities of these metals. The dust input of all these metals must therefore be regarded as high when compared with quantities already present in the vegetation.

Other trees behaved rather differently with respect to the three metals; for example, hawthorn and willow retained larger proportions of zinc and lead after washing than did oak, which may be

explained by the thicker, waxy cuticle of oak. Skarr *et al.* (*Nature*, **241**, 215; 1973) found that some bryophytes (which lack a waxy cuticle) absorb lead and store it in their nuclear membranes. Unfortunately, nothing is known of the uptake of these metals from the soil so that it is impossible to determine the relative importance of foliar absorption. Most of the matter washed from leaf surfaces accumulates in the superficial soil layers, as a result of which shallow rooting herbaceous species may be affected by soil toxicity more than deeper rooting trees.

A further study of dust accumulation, this time in a wetland ecosystem, is described by McLay (*Environ. Pollut.*, **5**, 173; 1973). In an area remote from industrial pollution in California, he examined a small alkaline lake where emergent *Scirpus californicus* grew around the edge and where *Lemna perpusilla* was abundant on the surface. Growth of *Lemna* was found to be significantly greater in the laboratory if washings from *Scirpus* stems were added to the lake water in which they were grown. McLay fails to demonstrate, however, that the stimulation is a result of additional nutrients from dust. It could quite easily be explained in terms of leaf exudates from *Scirpus*, either of an inorganic nature or as organic, growth promoting substances.

No smoke without fire

AN unusual line of research but one that may have environmental significance is the ecology of smoke particulates and charcoal residues from forest and grassland fires. E. V. and B. B. Komarek, who work at the Tall Timbers Research Station, Tallahassee, and T. C. Carlyle of the Gainesville Laboratory of the US Department of Agriculture, believe that airborne particles from such fires act as environmental cleansing agents. They have found from electron microscopy that the plant material which remains after burning has a varied porous structure and they suggest that the particulates could act as natural filters.

As a contribution to further studies of the part played by this material in

the atmospheric ecosystem, Komarek *et al.* have prepared an atlas of particulates collected from controlled fires (*Misc. Publ. Tall Timbers Research Station*, No. 3; 1973). Although the airborne material and the residues that remain on the ground vary from carbonized material to minerals, white ash, tars and gases, some of the original plant genera can be identified. Species of pine and hardwood trees, grasses and cultivated plants, for example, are represented in the atlas of 136 microphotographs. The figure here shows a cross section of a charred needle of spruce pine (*Pinus glabra*).

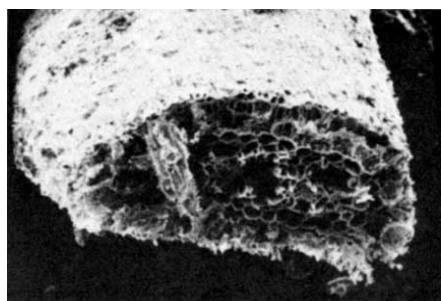
Breaking down bacterial messengers

from our
Molecular Genetics Correspondent

ALTHOUGH in most areas of nucleic acid synthesis studies of bacteria have led the way and research with eukaryotic systems is only now following, the processing of RNA is an exception; for the maturation of ribosomal RNA by cleavage from a large precursor is well established in eukaryotes and it is evident also that messenger RNA is initially transcribed in the nucleus in a form much larger than its eventual cytoplasmic size. Two studies reported in the first part of the December *Proceedings of the National Academy of Sciences* now provide a welcome balance by throwing some light on an enzymatic activity which may be involved in processing RNAs in bacteria.

Using the DNA of phage T7 as a template, Dunn and Studier and others also have shown previously that the messenger RNAs synthesised *in vitro* are derived by transcription from the beginning of the DNA to a point about 20% along it, whereas the mRNA found *in vivo* consists of five smaller species which seem to be derived by cleavage of a precursor corresponding to the *in vitro* messenger. The enzymatic activity which breaks the precursor down seemed to be very similar to ribonuclease III, one of the known ribonucleases of *Escherichia coli*. Taking advantage of a mutant strain of *E. coli* deficient in this enzyme, Dunn and Studier now report (*ibid.*, **70**, 3296; 1973) that this enzyme is implicated in both T7 mRNA cleavage and in rRNA cleavage.

In spite of the absence of a paragraph apparently omitted from the beginning of the "Results" section (this issue of the *Proceedings* appears with an apology from the editors for its delayed publication and lack of consistency in conventions followed in different papers), it seems that Dunn and Studier have examined the T7 RNAs produced in



normal and RNase III deficient strains of *E. coli* by gel electrophoresis. They find that the ribonuclease deficient strain the usual pattern of small RNAs is replaced by a large RNA identical with that usually transcribed *in vitro*. Extracts from *E. coli* strains containing ribonuclease III can cleave this large RNA to generate the messengers usually found *in vivo*.

Another maturation event, studied both by Dunn and Studier and also by Nikolaev, Silengo and Schlessinger (*ibid.*, 70, 3361; 1973), seem to take place in ribosomal RNA synthesis. In the RNase III deficient strain, synthesis of ribosomal RNA is slowed and the usual 16S and 23S sedimenting molecules are replaced by a precursor of 30S or $1.8 - 2.3 \times 10^6$ daltons (according to the different estimates), large enough to contain both sequences. Hybridisation experiments confirmed that the giant molecule competes with both the mature species. Cleavage *in vitro* with an extract of strains possessing RNase III showed that this activity can generate two molecules of about the size of 16S and 23S rRNAs.

The production of ribosomal RNA in *E. coli* is thus very similar to the pathway previously characterised in eukaryotic cells, although the eukaryotic precursor is some twice the combined size of the mature molecules, so that a great amount of additional material is degraded. It is not clear just how much larger the bacterial precursor is compared with the combined mature molecules. One puzzling feature of these results is that precursors only slightly larger than the 16S and 23S rRNAs have previously been described, a point mentioned in neither of the present articles. One model is to suppose that an initial cleavage generates the precursors found, which are then further degraded and modified to yield mature ribosomal RNAs.

That the cleavage of precursors to yield mature RNAs is found also with a phage messenger is, as well as its importance in excluding other mechanisms previously proposed for phage mRNA synthesis, important for its implication that this might occur with bacterial messengers. Any molecule of RNA possessing the specific sequence recognised by ribonuclease III will presumably be cleaved by the enzyme; future research will no doubt elucidate the recognition sequence and investigate its possible presence in bacterial messengers.

Another striking claim concerning the metabolism of RNA is made by Downey *et al.* (*ibid.*, 70, 3400; 1973), who report that a cytoplasmic RNA dependent RNA polymerase—in other words an RNA replicase—can be found in lysates of rabbit reticulocytes. The enzyme

seems to utilise RNAs as templates *in vitro* to direct incorporation of labelled triphosphates into an acid precipitable product. The enzyme proved inactive when provided with DNA as primer.

The presence of an RNA replicase in the cytoplasm would add a new level of control to expression and could, for example, account for production of haemoglobin after synthesis of mRNA has ceased (instead of postulating that the haemoglobin mRNA is stable). But before this conclusion can be accepted it will be necessary to categorise rigorously the product of the reaction by qualitative means such as hybridisation assays instead of the only preliminary quantitative assay of total nucleotide incorporation. And of course, it is necessary to bear in mind frequent demonstrations of recent years that activities *in vitro* make few implications about enzyme roles *in vivo*.

Function of chloroplast DNA and its replication

from a Correspondent

ALTHOUGH the presence of DNA in chloroplasts has been recognised for more than a decade, surprisingly little is known about its function. Measurements of the molecular weight of the chloroplast genome generally give values of approximately $1-2 \times 10^6$ daltons. This is sufficient to code for several hundred proteins, yet isolated chloroplasts have only been shown to synthesise the larger subunit of the fraction 1 protein and two membrane-associated proteins (Blair and Ellis, *Biochem. J.*, 127, 42P; 1972; Ellis and Forrester *ibid.*, 130, 28P; 1972).

It seems probable that many chloroplast enzymes are coded for by nuclear genes. The genetics of the fraction 1 protein, which catalyses the carboxylation of ribulose diphosphate, are particularly interesting. The gene for the larger subunit of the protein shows cytoplasmic inheritance and is presumably located in the chloroplast whereas the gene controlling the smaller subunit shows Mendelian inheritance and is therefore in the nucleus (Kawashima and Wildman, *Biochim. biophys. Acta*, 262, 42; 1972; Chan and Wildman, *ibid.*, 277, 677; 1972). Another part of the chloroplast DNA codes for the 1.1×10^6 and 0.5×10^6 dalton RNA components of the chloroplast ribosomes (Scott and Smillie, *Biochem. biophys. Res. Commun.*, 28, 598; 1967) and these RNAs have been shown to be synthesised inside chloroplasts (Hartley and Ellis, *Biochem. J.*, 134, 249; 1973).

On the other hand DNA-RNA hybridisation studies with higher plant systems have previously suggested that

a large number of genes for chloroplast ribosomal RNA are also present in the nucleus although there are alternative explanations of these results (Tewari and Wildman, *Proc. natn. Acad. Sci. U.S.A.*, 59, 569; 1968; Ingle *et al. Symp. Soc. exp. Biol.*, 24, 303; 1970). Experiments by Scott now suggest that at least in *Euglena* chloroplast ribosomal RNA may be coded for only by chloroplast DNA (*J. molec. Biol.*, 81, 327; 1973).

Of the total DNA in light-grown *E. gracilis* cells, between 5 and 6% is present in chloroplasts. There are about ten chloroplasts in each cell and each organelle contains 40-80 copies of the chloroplast DNA which has a molecular weight between 1.8×10^6 and 0.7×10^6 daltons. DNA isolated from the chloroplasts has an average buoyant density in caesium chloride of 1.686 g cm^{-3} , which distinguishes it from the nuclear DNA with an average density of 1.707 g cm^{-3} . When RNA from chloroplast ribosomes is hybridised to denatured chloroplast DNA immobilised on nitrocellulose filters, the saturation values give an estimate of 6% of the DNA coding for chloroplast rRNA. These genes are clustered on the DNA and have a buoyant density of 1.696 g cm^{-3} . There are separate cistrons for the larger and smaller RNAs with between three and six copies of each cistron per chloroplast genome depending on which value for the size of the genome is correct. This works out at 2,400 genes for chloroplast rRNA per cell. By contrast there are 1,000 genes, buoyant density 1.716 g cm^{-3} , coding for the RNA of the cytoplasmic ribosomes. Such a multiplicity of rRNA genes is commonly found in eukaryotes and is consistent with a high metabolic demand for ribosome synthesis.

Euglena cells grown heterotrophically in the dark do not develop chloroplasts. When DNA from dark-grown cells is challenged with chloroplast rRNA, Scott found that although there is some hybridisation this represents a non-specific reaction. Heating the 'hybrids' dissociates the DNA-RNA duplexes and the melting profile thus obtained affords a measure of the degree of base pairing involved. The results clearly show that the duplexes have a much lower thermal stability than authentic rRNA-rDNA hybrids.

The conclusion is that DNA from dark-grown cells (largely nuclear DNA) does not contain a large number of genes for chloroplast rRNA. Presumably there must be at least one copy of the chloroplast DNA present in dark-grown cells but even several copies could have remained undetected in these experiments. For this reason the possibility that there are a very small number of chloroplast rRNA genes in the nucleus cannot yet be ruled out.

Cosmic Far Ultraviolet Background

Arthur Davidsen, Stuart Bowyer and Michael Lampton

Space Sciences Laboratory and Astronomy Department, University of California, Berkeley, California 94720

Observations of a diffuse ultraviolet background at high galactic latitude might reveal the existence of an intergalactic medium. Although existing results provide only weak constraints, current techniques are sufficient to set interesting limits on its density and temperature.

INTEREST in the diffuse far ultraviolet background stems mainly from its relevance to the widely held view that intergalactic space may be filled with a dilute gas containing the majority of the mass of the universe. Observations which might reveal the existence of the intergalactic medium (IGM) have been attempted at radio, optical, far ultraviolet and X-ray wavelengths (see ref. 1 for a recent review). If the IGM has a density comparable to the critical density required to close the universe, then 21 cm radio observations and optical observations of QSO spectra imply the gas is highly ionised and therefore $T \gtrsim 10^4$ K. On the other hand observations of the diffuse X-ray background show that the temperature of such a gas is probably less than about 3×10^8 K. In the lower part of this acceptable range of temperatures, the IGM would radiate primarily in the far ultraviolet. Alternatively, the high degree of ionisation of the gas may be maintained by a large ultraviolet flux from some as yet unidentified source. Thus, the far ultraviolet region of the spectrum deserves considerable attention in the effort to determine the density and temperature of the IGM.

Since the first report² of an observation of the diffuse far ultraviolet background, there have been several measurements attempted, both longward and shortward of Lyman α . As the reported intensities range over nearly three orders of magnitude, a critical evaluation is required before the observations may be interpreted in terms of limits on the physical state of the IGM. Here we discuss the expected intensities of various possible components of the far ultraviolet background and review the relevant observations.

We conclude that existing results do not place interesting constraints on the density of the IGM. Current techniques and instrumentation for far ultraviolet astronomy are, however, sufficient to achieve vastly improved limits, but new observations are required to determine whether the IGM can be detected in the far ultraviolet or whether the extragalactic component of the background is masked by radiation with a more local origin.

There are several contributors to the far ultraviolet background radiation in addition to the possibility of emission from the IGM. The extragalactic background will include a contribution from galaxies and QSOs. A galactic background will be present which will consist of direct stellar radiation and a truly diffuse component resulting from starlight scattered by interstellar dust. Locally there will be contributions from the zodiacal light as well as airglow emissions from the Earth's geocorona. In what follows, we discuss briefly what is known, either theoretically or observationally, about each of the components of the far ultraviolet background.

Intergalactic medium

Attempts³ to observe Lyman α absorption in the optical spectra of large redshift QSOs have led to the conclusion that the IGM is either remarkably empty, or it was highly

ionised at $z \geq 2$. Such a high degree of ionisation implies temperatures greater than about 10^4 K. Kurt and Sunyaev⁴ first discussed Lyman α radiation from recombinations in the IGM. Emission in this line is maximised at $T \approx 2 \times 10^4$ K, where an intensity of 4×10^{-10} erg (cm² s sr Å)⁻¹ just longward of Lyman α is produced in a uniform universe having the critical density and with the Hubble constant $H_0 = 50$ km s⁻¹ Mpc⁻¹. The best current estimate⁴ of the deceleration parameter in a Friedmann model of the universe is $q_0 = 1 \pm 1$ and this yields an intensity of 1.6×10^{-9} erg (cm² s sr Å)⁻¹.

Silk and Tarter⁵ have considered a clumpy IGM, consisting of clouds of ionised gas which are distributed similarly to galaxies. In such a model, it is possible to gravitationally bind groups and small clusters of galaxies, as well as the universe, without violating limits on the ultraviolet and soft X-ray backgrounds. Since the clumpiness increases the emissivity of the IGM per unit mass, a substantial flux of Lyman α recombination radiation may be produced even at $T \gg 2 \times 10^4$ K.

The cosmic Lyman α flux is also relevant to the arguments which lead to the conclusion that a dense, neutral IGM cannot exist. Although the QSO observations already mentioned provide extremely strong limits on neutral hydrogen (n_H ($z=2$) $< 1.5 \times 10^{-11}$ cm⁻³), this result depends on the assumption that QSO redshifts are cosmological. Even with this assumption, these data provide information only about a distant epoch. Since the cosmological distance assumption is questioned by some astronomers and since the ionisation of the IGM at the present epoch may differ from that at $z \approx 2$, the limits on neutral hydrogen obtained through 21 cm observations of low redshift objects are still of interest. These observations¹ give $n_H/T_E < 1.8 \times 10^{-8}$ cm⁻³ K⁻¹, where T_E is the excitation temperature of the 21 cm line. The principal unknown contribution to T_E comes from recombination Lyman α in the IGM. Thus an upper limit on the Lyman α flux is required to set a limit on T_E and thus on n_H . An indirect limit of $T_E < 12$ K has been set by Field¹. A direct observational confirmation of this requires the establishment of an upper limit of 4×10^{-10} erg (cm² s sr Å)⁻¹ for the cosmic Lyman α flux.

In addition to ionised hydrogen, the IGM is generally believed to contain about 10% helium which was produced in the primeval fireball. At a temperature of about 10^5 K the IGM will thus radiate strongly in the HeII λ 304 Å recombination line. Radiation of this wavelength, originating at epochs with $z \approx 2.5$ to 3.0, will be redshifted into the observable spectral region around 1,100 Å. Kurt and Sunyaev^{2,6} first pointed out that because the density in an expanding universe varies as $(1+z)^3$ and the intensity of a recombination line varies as the square of the density, measurement of the redshifted helium radiation provides an even more sensitive probe of the IGM than that provided by the hydrogen radiation. Thus observations shortward as well as longward of Lyman α may yield information on the thermal history of the IGM back to epochs where the most distant QSOs have been observed.

Galaxies and QSOs

The possibility that the IGM is photoionised by far UV radiation from QSOs and galaxies has been considered by several authors⁷⁻¹¹. Rees and Setti⁹ estimate that QSOs alone

would produce a Lyman limit intensity of about 4×10^{-11} to 15×10^{-11} erg (cm² s sr Å)⁻¹. They also suggest a similar contribution could be made by an extrapolation of the diffuse X-ray background spectrum to ultraviolet wavelengths. Such a flux would be sufficient to maintain a high degree of ionisation, consistent with the Gunn-Peterson result, in an IGM whose density is $n \sim 10^{-7}$ cm⁻³. Rees and Setti conclude that an IGM with the critical density could be sufficiently photoionised by a far ultraviolet background whose intensity is $\sim 2 \times 10^{-9}$ erg (cm² s sr Å)⁻¹. The existence of such a universal ultraviolet flux might have profound implications beyond its effect on the IGM.

A suggestion that normal galaxies are strong emitters of far ultraviolet radiation has recently been made by Hills¹². According to this work, ultraviolet dwarfs with temperatures of about 2×10^5 K emit enough ultraviolet photons to maintain a high degree of ionisation in an IGM having the critical density. This radiation, however, is strongly concentrated beyond the Lyman limit, and the intensity in the observable region at approximately 1,300 Å would be only about $\sim 5 \times 10^{-12}$ erg (cm² s sr Å)⁻¹. On the other hand, if such hot stars were numerous in young galaxies at large z , their contribution to the intensity at approximately 1,300 Å might be substantially larger.

Although the intensity of the cosmic far ultraviolet background is not predictable, it is clear that a sensitivity of $\leq 10^{-9}$ erg (cm² s sr Å)⁻¹ should allow some interesting conclusions to be drawn. An extragalactic background of this intensity, however, might be overwhelmed by galactic and even more local sources of ultraviolet radiation, thereby forever precluding investigation of the IGM at ultraviolet wavelengths. We turn now to a consideration of these other components of the ultraviolet background.

Galactic starlight

It is clear that observations made with detectors with large fields of view will include a spurious flux component arising from point sources whose space density is sufficiently large. The mean intensity of direct starlight in the solar neighbourhood at approximately 1,400 Å is about 1×10^{-7} erg (cm² s sr Å)⁻¹ according to calculations by Habing¹³. Other calculations¹⁴ suggest a variation of a factor of about ten in intensity of direct starlight from galactic plane to pole. Thus, observations made with large field detectors may be expected to yield $I(1,400 \text{ Å}) \geq 10^{-7}$ erg (cm² s sr Å)⁻¹ to within a factor of ten. This is consistent with the observations discussed below.

To observe the cosmic background, this pseudo background must be substantially reduced by using a small field of view. As the field of view is made smaller, sources of progressively higher space density are eliminated. This has a particularly important effect on far ultraviolet observations, as the relatively low density objects which may be completely eliminated with a small field of view are just those hot stars which contribute most to the ultraviolet flux measured with a large field detector. Of course, as the field of view is reduced, so is the sensitivity of the detector to diffuse radiation. There must therefore be an optimum field of view which is just small enough to eliminate a substantial contribution from stars.

For a field of view of 0.1 square degrees, only stars of spectral type F and later are numerous enough to contribute to the diffuse flux at high galactic latitude, but such stars are intrinsically weak in the far ultraviolet. If a telescope is oriented to avoid stars brighter than $V=10$, we expect the stellar contribution to the background will be less than approximately 10^{-11} erg (cm² s sr Å)⁻¹, substantially below the predicted extragalactic flux. Similar conclusions have been reached by Kurt and Sunyaev⁶ and by Smirnov¹⁵. With this field of view the ultraviolet dwarfs discussed above will not be sufficiently numerous to produce a significant diffuse component either.

Diffuse galactic light

Extinction of far ultraviolet radiation in the galaxy is due to interstellar grains. Some models of these grains, such as dielectric particles and Platt particles, predict high albedos. If the extinction is mainly due to scattering, a diffuse galactic light will exist, whose origin is in starlight. If the albedo is very high, the energy density of this diffuse light may be comparable to that of the direct starlight. The intensity of diffuse light will vary with direction in the Galaxy in a way which depends on the distribution of hot stars, the distribution of the dust, the albedo, and the phase function for the scattering. For a simple model of the galaxy in which the stars and dust are similarly distributed in plane parallel layers, the variation of intensity of diffuse light with galactic latitude has been calculated by Van de Hulst and de Jong¹⁴. Taking the grain parameters predicted by Gilra's models¹⁶ and a galactic half optical thickness of 0.2, we find from Van de Hulst and de Jong's work that the diffuse light at the pole is about 0.5% of the total intensity (including starlight) near the galactic plane. This intensity¹⁷ is about 5×10^{-7} erg (cm² s sr Å)⁻¹ at 1,400 Å. Thus, the diffuse galactic light predicted at the pole in this model is 2.5×10^{-9} erg (cm² s sr Å)⁻¹, which is somewhat larger than the expected Lyman α flux from the IGM.

If the plane-parallel model were applicable, the diffuse galactic light would make a measurement of the cosmic far ultraviolet background difficult. It has been shown, however, that this model for the distribution of interstellar dust is inadequate. A wide variety of studies¹⁸ suggest that the interstellar reddening $E(B-V)=0.00$ for galactic latitudes greater than $b=50^\circ$. This result may easily be reconciled with the usual cosecant b dependence of the plane-parallel galaxy models when it is realised that the dust is concentrated in clouds. At low latitudes, where a line of sight intersects many clouds, the uniform distribution assumption is not inappropriate but at high latitudes most lines of sight simply do not intersect a dust cloud. Thus, for most directions above $b=50^\circ$ no diffuse galactic light is expected and therefore the cosmic UV background will not be masked.

Zodiacal light

The zodiacal light results from solar radiation scattered by interplanetary dust and is one of the principal contributors to the sky brightness at visual wavelengths. Because of the sharp decrease of the solar flux toward short wavelengths, however, it may be expected that the zodiacal light will not contribute much to the far ultraviolet background. Thus, Kurt and Sunyaev⁶ concluded that the intensity of the zodiacal light at 1,300 Å is less than 2×10^{-12} erg (cm² s sr Å)⁻¹. On the other hand, Lillie¹⁹ presented preliminary evidence that the zodiacal light spectrum turns up below 2,500 Å. His data suggested a continued steep rise toward the shortest wavelengths observed (about 1,680 Å). More recent data (C. F. Lillie, unpublished), obtained with the ultraviolet spectrometer aboard Apollo 17, show that the spectrum turns down again below 2,000 Å. In fact, the relative intensity of the zodiacal light, normalised to the Sun, is now thought to be the same at about 1,680 Å and 4,250 Å. Thus, the conclusion of Kurt and Sunyaev that this component is negligible in the far ultraviolet is probably correct. In any case, a zodiacal light flux would certainly show a concentration toward the ecliptic. The zodiacal light, therefore, probably does not present any important limitation on the measurement of a cosmic far ultraviolet background.

Airglow

Probably the most important limitation on rocket observations of the diffuse far ultraviolet background is the contribution of line emission originating in the geocorona. By far the strongest of these lines is Lyman α $\lambda 1,216$, whose intensity in the night sky is $\sim 10^9$ photons (cm² s sr)⁻¹. No other

lines have been observed, but it is possible that the OI $\lambda 1,304$ triplet and OI $\lambda 1,356$ will be significant at the flux levels under discussion here. If these lines have intensities of as little as 1 rayleigh, their contribution to the diffuse background measured with a typical photometer having a bandpass of about 200 Å is approximately 10^{-8} erg (cm² s sr Å)⁻¹. At present, such an intensity cannot be ruled out. Just as a small field of view is required to eliminate discrete spatial points (stars) which produce continuous spectral contributions, so is a narrow bandpass required to eliminate discrete spectral points (airglow lines) which produce a continuous spatial contribution, and attempts to observe the cosmic far ultraviolet background from rockets will require at least moderate resolution. Observations from high altitude satellites or interplanetary probes will not be limited in this way.

Observations

In spite of several attempts to measure the intensity of the diffuse ultraviolet background just longward and shortward of Lyman α , the subject remains in a state of confusion. Results of all previous measurements, displayed in Figure 1, range over two and a half orders of magnitude. The large range of the positive detections probably results from the large fields of view employed and the distribution of hot stars in the Galaxy.

The first attempt to measure the far ultraviolet back-

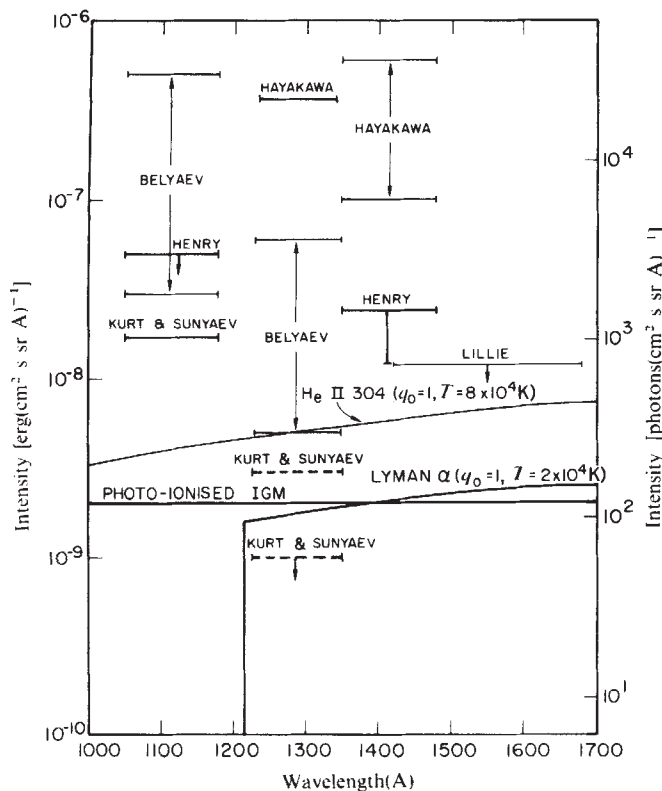


Fig. 1 Observed and predicted intensities of the diffuse far ultraviolet background. The measurements are discussed in the text. The lowest intensities, due to Kurt and Sunyaev, are contradicted by later results. The Lyman α flux from recombinations IGM with $q_0 = 1$ and $T = 2 \times 10^4$ K (assumed constant with Z) is shown. A similar flux due to He II 304 Å recombinations at large Z is also shown. Also presented is an estimate of the flux which is necessary to produce a sufficient degree of photoionisation in an IGM having the critical density. Local and Galactic components of diffuse light are probably weak enough at high latitude to allow detection of a cosmic flux of approx 10^{-9} erg (cm² s sr Å)⁻¹. A sensitivity at this level can be achieved with current instrumentation.

ground in the neighbourhood of Lyman α was made by Kurt and Sunyaev² using a detector aboard the interplanetary probe Venera-3. With a field of view of approximately 300 square degrees and a bandpass from $\lambda 1,225$ to $1,340$ Å, they detected no signal above that due to the cosmic ray background. Kurt and Sunyaev thus obtained an upper limit $I_\lambda \lesssim 5 \times 10^{-22}$ erg (cm² s sr Hz)⁻¹ or $I_\lambda \lesssim 1.0 \times 10^{-9}$ erg (cm² s sr Å)⁻¹. This result is generally considered suspect, because Kurt and Sunyaev²⁰ later reported a detection, with the same instrument, of the hot stars near the galactic plane at an intensity which is two orders of magnitude below the theoretically expected value^{13,21} and the value observed by Hayakawa, Yamashita, and Yoshioka¹⁷ in the same direction.

Another measurement in the region $\lambda 1,225$ to $1,340$ Å was reported by Belyaev *et al.*²² with an instrument aboard Venera-5 and Venera-6. These authors also measured the background in the range $\lambda 1,050$ to $1,180$ Å. The fields of view were approximately 120 and 0.4 square degrees for the shorter and longer wavelengths, respectively. Intensities obtained in the $\lambda 1,225$ to $1,340$ Å interval varied from about 2×10^{-8} to about 5×10^{-8} erg (cm² s sr Å)⁻¹ near the galactic plane and an upper limit of $I_\lambda \lesssim 5 \times 10^{-9}$ erg (cm² s sr Å)⁻¹ was obtained in an undetermined direction. The results were of very low statistical accuracy because of the small field of view, a rather small collecting area (about 10 cm²), and a high cosmic-ray background. In the interval $\lambda 1,050$ to $1,180$ Å, Belyaev *et al.* obtained intensities ranging over about 3×10^{-8} to 65×10^{-8} erg (cm² s sr Å)⁻¹. The authors attribute the much higher intensities observed in this band to the larger field of view.

Hayakawa, Yamashita and Yoshioka¹⁷ have measured the background intensity in the range $\lambda 1,350$ to $1,480$ Å with a detector whose field of view was 20 square degrees. Their results ranged from about 5×10^{-7} erg (cm² s sr Å)⁻¹ near the galactic plane smoothly down to about 1×10^{-7} erg (cm² s sr Å)⁻¹ at $b \approx 40^\circ$. These authors also obtained an upper limit to a possible extragalactic intensity of $\leq 1.7 \times 10^{-8}$ erg (cm² s sr Å)⁻¹ on the basis of an analysis of the latitude variation in terms of the scattering properties of interstellar grains. This analysis was subsequently revised²³ with the result that no information on the extragalactic component was obtained.

Using photometers aboard OAO-2, Lillie²⁴ has obtained an upper limit on the diffuse intensity at high galactic latitude in a 260 Å band centred at $1,550$ Å. With the extremely small field of view (about 0.02 square degrees) of this instrument, the contribution due to hot stars was eliminated. Unfortunately Lillie observed no signal above the dark counting rate because of the low throughput of the instrument. His resultant upper limit was 1.2×10^{-8} erg (cm² s sr Å)⁻¹.

Henry²⁵ has reported measurements of the high latitude background in the range $\lambda 1,050$ to $1,180$ Å and $\lambda 1,350$ to $1,475$ Å, made with a field of view of approximately 100 square degrees. In the longer wavelength range, he obtained an intensity of about 2×10^{-8} erg (cm² s sr Å)⁻¹. Shortward of Lyman α , he obtained only the upper limit (I_λ 1,100 Å) $\lesssim 3 \times 10^{-8}$ erg (cm² s sr Å)⁻¹ since the measured signal was largely due to residual sensitivity of his detector to Lyman α radiation.

Intensities

It seems likely that most of the large range of intensities in Fig. 1 is due to the varying number of hot stars with galactic latitude. These are the main contributors to the intensity measured with a large field of view. In addition there is undoubtedly an important contribution from diffuse galactic light at low latitudes. Except for the results of Kurt and Sunyaev, the lowest intensities were obtained with the detectors having the smallest fields of view. These results were upper limits, and are limited by the sensitivity of the instruments. Unfortunately, these limits are not yet strong

enough to place important constraints on the density of the IGM. A sensitivity of $\lesssim 10^{-9}$ erg (cm² s sr Å)⁻¹, achievable with current instrumentation, would provide an interesting limit. Local and galactic components of diffuse far ultraviolet radiation are probably weak enough at high galactic latitude to allow detection of a cosmic intensity of this magnitude.

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Primary structure of a mouse myeloma cell initiator transfer RNA

Peter W. Piper & Brian F. C. Clark

Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH

The primary structure of cytoplasmic tRNA_f^{Met} (the initiator tRNA) from mouse P3 myeloma cells is given and its most unusual features are discussed.

THE initiation step of mammalian protein synthesis involves a unique methionine-accepting transfer RNA. This initiator tRNA, which is usually denoted tRNA_f^{Met} in respect of its ability in its charged form to function as a substrate for *E. coli* methionyl-tRNA_f^{Met} transformylase, serves to donate the amino acid which is to form the N-terminus of the newly synthesised polypeptide¹⁻³. In prokaryotes, as well as within the cell organelles of eukaryotes, N-formyl-methionyl-tRNA_f^{Met} functions as the initiating species of tRNA, whereas in the cytoplasm of eukaryote cells no formylation of methionyl-tRNA_f^{Met} occurs before it provides the first amino acid of nascent polypeptides. The methionyl-tRNA_f^{Met} of both bacteria and higher organisms is distinguished from other aminoacyl-tRNAs engaged in protein synthesis by its inability to participate in polypeptide elongation and thereby provide its amino acid moiety for the internal positions of a protein amino acid sequence. Methionine residues which are not N-terminal in the protein are contributed by another methionine-accepting tRNA species, denoted tRNA_m^{Met}, which operates in protein chain elongation but not in initiation.

We have investigated the structure of a mammalian initiator tRNA in the hope that it might be possible to relate a special tRNA structure to the unique role played by this molecule in protein biosynthesis. We report here the primary structure of the cytoplasmic tRNA_f^{Met} of mouse P3 myeloma cells and discuss the most unusual features of the nucleotide sequence. To date only two other complete mammalian tRNA sequences have been reported^{4,5}.

The ³²P-labelled mouse P3 myeloma tRNA_f^{Met} was isolated from the cytoplasm of cells cultured in the presence of ³²P-phosphate⁶ and was purified by reversed-phase chromatography on an RPC-5 column⁷. The structural similarity of tRNA_f^{Met} isolated from different mammalian sources is dis-

cussed in the following paper by Simsek, Boissard, Petrisant and RajBhandary⁸. Fingerprints of the oligonucleotides obtained on digestion of mouse myeloma tRNA_f^{Met} with T₁ RNase and pancreatic RNase are reproduced in Fig. 1. A comparison of these fingerprints with those in the accompanying paper serves to illustrate the close similarity between the nucleotide sequences of mouse myeloma initiator tRNA and other mammalian tRNA_f^{Met} species which have been investigated. Oligonucleotide sequencing was performed using the techniques developed by Sanger and coworkers for the structural analysis of ³²P-labelled RNA, as detailed by Barrell⁹. The purification of the myeloma initiator tRNA and the determination of its nucleotide sequence will be described in detail elsewhere.

The complete primary structure of mouse myeloma cell cytoplasmic tRNA_f^{Met} is presented in Fig. 2a arranged in a clover leaf. We consider that the two most noteworthy features of the structure are the loop IV (or -G-T-Ψ-C-loop) sequence together with the unusual presence of a cytidine residue immediately preceding the anticodon in the anticodon loop. We have obtained no evidence for any structural heterogeneity of mammalian cytoplasmic initiator tRNA so there is apparently only a single tRNA_f^{Met} present in the cytoplasmic fractions derived from the cell line which we have studied. This is in contrast to *Escherichia coli* which contains two tRNA_f^{Met} sequences which differ in the nucleotide found at a single location¹⁰.

The outstanding characteristic of the mammalian initiator tRNA structure is its considerable similarity to the sequence of yeast tRNA_f^{Met} which was determined by Simsek and RajBhandary¹¹. There is also considerable, although not quite so extensive, homology with the nucleotide sequence of *E. coli* tRNA_f^{Met} (ref. 10). The similarity between the primary structures of tRNA_f^{Met} isolated from *E. coli*, yeast and mouse myeloma cell cytoplasm is shown in Fig. 2b. The special role of initiator tRNA apparently imposes structural constraints such that tRNA_f^{Met} nucleotide sequences have only diverged to a limited extent during evolution. Much of the tRNA_f^{Met} sequence conservation occurs in the regions of the clover

leaf where the nucleotide sequences of tRNAs which charge with different amino acids generally show the greatest variation¹². All three molecules compared in Fig. 2b are substrates for both *E. coli* methionyl-tRNA synthetase and *E. coli* methionyl-tRNA transformylase. The tRNA^{Met} structural features which are possibly involved in *E. coli* methionyl-tRNA synthetase recognition are discussed in the following paper⁸. The conserved regions of nucleotide sequence in Fig. 2b might reflect features of tRNA^{Met} structure which are necessary for *E. coli* transformylase recognition.

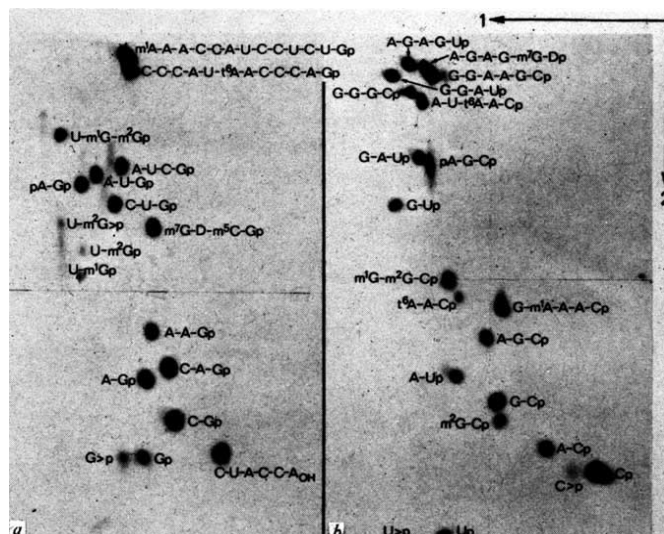


Fig. 1 Autoradiograms of two-dimensional fractionations of RNase digests of ³²P-labelled mouse myeloma tRNA^{Met}. Separations of the oligonucleotides in both a T₁ RNase digest (a) and a pancreatic ribonuclease digest (b) are shown. The first dimension (1) was electrophoresis at pH 3.5 on cellulose acetate and the second dimension (2) was electrophoresis on DEAE-cellulose paper in 7% formic acid.

The unusual unpaired base sequence -A-U-C-G-m¹A-A-A-, hitherto found only in the loop IV region of yeast initiator tRNA^{Met}, is reproduced exactly in the mouse myeloma tRNA^{Met} structure (Fig. 2a) and has apparently been conserved during eukaryote evolution. In general both yeast and mammalian tRNAs have pyrimidines as the first and last unpaired bases of loop IV of the tRNA clover leaf. As far as is known only in eukaryote initiator tRNAs are these pyrimidines replaced by adenosine residues⁸. In contrast *E. coli* tRNA^{Met}, the only prokaryotic initiator tRNA for which the sequence is known¹⁰, contains a much more conventional sequence in loop IV (-T-Ψ-C-A-A-U-). It would be interesting to know whether the considerable differences in the loop IV sequences of prokaryote and eukaryote tRNA^{Met} structures reflect a different mode of initiator tRNA binding to ribosomes in prokaryotes and eukaryotes. Recent evidence indicates that the initial binding of initiator tRNA to mammalian 40S ribosome subunits during the formation of initiation complexes may be independent of template^{13,14} and therefore suggests that the initiation site on mammalian mRNA may be selected by the anticodon of tRNA^{Met} bound to the subunit.

The mouse myeloma tRNA^{Met} has cytosine immediately preceding the anticodon in place of the uracil residue hitherto universally found in this position in tRNA sequences. Virtually nothing is known at present about the molecular geometry of different anticodon loop primary structures, and therefore the significance of this base substitution remains unclear.

Since the methionine tRNA which participates in poly-

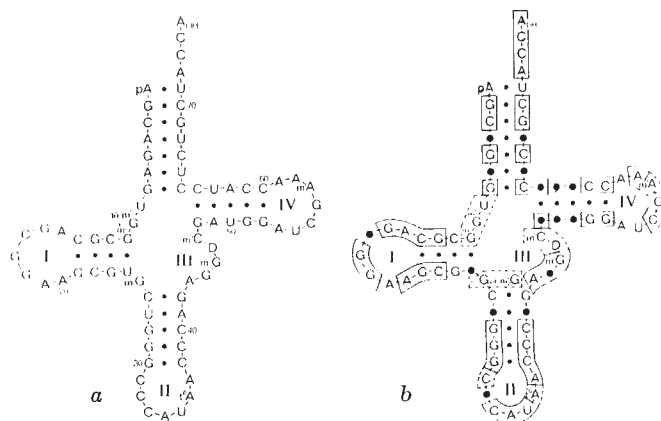


Fig. 2 a, The nucleotide sequence of mouse myeloma cell cytoplasmic initiator tRNA arranged in the clover leaf pattern. The Roman numerals I-IV identify the loops which are referred to in the text. b, An illustration of the similarities between the nucleotide sequences of the formylatable methionine-accepting tRNA species of mouse myeloma cell, yeast and *E. coli*. The clover leaf is drawn with those nucleotides which are common to both the myeloma and yeast tRNA^{Met} sequences inserted in their appropriate positions. Regions of adjacent nucleotides common to the mammalian and *E. coli* initiator tRNA sequences are enclosed in boxes, the dotted lines indicating where these two sequences differ only with regard to base modification.

peptide elongation in mammalian cells, tRNA^{Met}, is aminoacylated *in vivo* by the same methionyl-tRNA synthetase as the initiator tRNA, it was pertinent to investigate whether a eukaryote tRNA^{Met} would also have -A-U-C-G-m¹A-A-A- in loop IV. A preliminary analysis of the primary structures of the two major isoaccepting species of mouse myeloma cell tRNA^{Met} has indicated that they possess different nucleotide sequences. The loop IV sequences of these two tRNAs are -T-Ψ-C-G-m¹A-A-C- and -T-Ψ-C-G-m¹A-U-C- (P. W. P., unpublished results), and therefore resemble those found in the majority of tRNAs of known primary structure (including *E. coli* tRNA^{Met}). This is a further indication that the loop IV of mammalian initiator tRNA may differ significantly in structure from the loop IV of mammalian tRNAs which function in polypeptide elongation.

Only information on the three-dimensional folding of the polynucleotide chains of tRNA molecules will indicate whether the constant features near the anticodon in initiator tRNAs reflect an anticodon loop structure which is unique to tRNA^{Met} species. A special anticodon loop structure only found in initiator tRNAs might be a prerequisite for *E. coli* transformylase recognition and may also be responsible for the unexplained anomaly whereby tRNA^{Met} of *E. coli*¹⁵, yeast¹⁶ and mouse ascites tumour cells² has the ability to recognise both the codons ApUpG and GpUpG and to thus exhibit code degeneracy or 'wobble'¹⁷ at the third base (3' end) of the anticodon. In contrast the tRNAs which function in polypeptide elongation produce the degeneracy of the genetic code by ambiguous reading of the template triplet or 'wobble' at the first base (5' end) of the anticodon.

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Nucleotide sequence of rabbit liver and sheep mammary gland cytoplasmic initiator transfer RNAs

Mehmet Simsek & Uttam L. RajBhandary

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

M. Boissard & Guy Petrissant

Laboratoire de Physiologie de la Lactation, Centre National de Recherches Zootechniques, Jouy-en-Josas

The Nucleotide sequence of rabbit liver and sheep mammary gland cytoplasmic initiator tRNAs is identical to that of cytoplasmic initiator tRNA of mouse myeloma P3 cells.

THE first elucidation of the nucleotide sequence of a eukaryotic initiator tRNA was described recently¹. It was shown that this yeast cytoplasmic tRNA possessed two striking features which distinguished it from all tRNAs of known sequence, including the *Escherichia coli* initiator tRNA. These were absence of the sequence G-T-ψ-C-G(A)-present in loop IV of most tRNAs and its replacement by G-A-U-C-G-; and presence of A as the last nucleoside of this loop instead of U or C as in other tRNAs. Further studies²⁻⁵ have shown that both features are common also to mammalian cytoplasmic initiator tRNAs and that the entire sequence of loop IV of yeast and mammalian cytoplasmic initiator tRNAs are identical: A-U-C-G-m'A-A-A-. Studies on a plant cytoplasmic initiator tRNA have yielded essentially similar results except that the sequence of loop IV in this case is A-U*-C-G-m'A-A-A- (U*, modified U; Ghosh, Ghosh, G.P. and U.L.R., manuscript in preparation). These findings suggest that the two unusual features described above may be common to all eukaryotic cytoplasmic initiator tRNAs.

We summarise here the results of our work on the total nucleotide sequence of rabbit liver cytoplasmic initiator tRNA⁶ and also provide evidence that the sequences of rabbit liver and sheep mammary gland⁷ cytoplasmic initiator tRNAs may be identical. The sequence derived for the rabbit liver cytoplasmic initiator tRNA is identical to that deduced independently by Piper and Clark⁸ for the corresponding tRNA of mouse P3 myeloma cells. Thus, the nucleotide sequences of cytoplasmic initiator tRNAs from three mammalian sources which have diverged significantly during evolution⁹ have remained invariant. Details of this work will be described elsewhere.

Rabbit liver cytoplasmic initiator tRNA

The sequence of a fragment of thirty nucleotides obtained by cleavage of tRNA at the phosphodiester bond adjacent to m²G has been reported previously⁸. The remainder of the tRNA molecule was sequenced by an analysis of fragments obtained by partial digestion of the tRNA with pancreatic RNase. The sequence is identical to that of mouse P3 myeloma cell initiator tRNA reported in the preceding paper.

Comparison of nucleotide sequences

The following lines of evidence strongly suggest that the sequences of rabbit liver and sheep mammary gland cytoplasmic initiator tRNAs are identical. (1) Modified nucleoside composition analyses using the technique of Randerath and Randerath¹⁰ show that these two tRNAs contain the same modified nucleosides. The absence of m²G which is present in the yeast cytoplasmic initiator tRNA and the presence instead of two mol of m²G per mol of tRNA², one at G₁₀ and the other at G₂₈, is particularly noteworthy. (2) Fingerprints of complete T₁-RNase digests of these tRNAs are identical (Fig. 1). (3) Fingerprints of complete pancreatic RNase digests of these tRNAs are identical (Fig. 2). The corresponding spots from each of these fingerprints, Fig. 1 (a against b) and Fig. 2 (a against b), have been analysed further by 5'-end group analysis and partial digestion with snake venom phosphodiesterase. The patterns of radioactive spots obtained after partial snake venom phosphodiesterase treatment and electrophoresis on DEAE-cellulose paper¹² were identical for each of the corresponding spots. These results show that the series of fragments produced by T₁-RNase and by pancreatic RNase on rabbit liver and sheep mammary gland initiator tRNAs possess identical sequences.

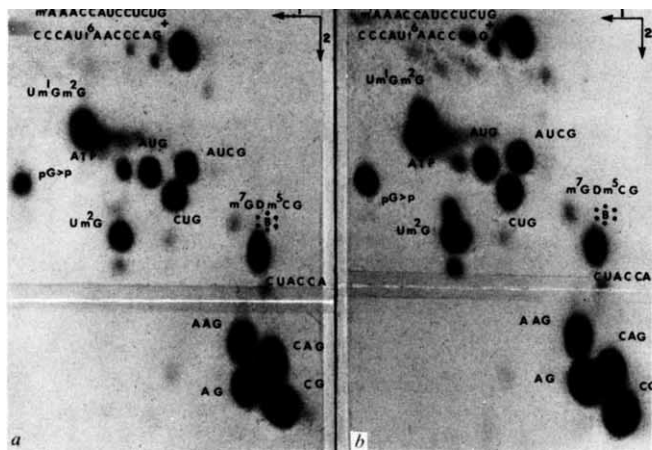


Fig. 1 Autoradiographs of 5-³²P-labelled oligonucleotides obtained from T₁-RNase digests from rabbit liver (a) and sheep mammary gland (b) cytoplasmic initiator tRNAs. The procedure used was identical to that described before², except that 0.01 unit of *E. coli* alkaline phosphatase was used instead of 5 units. Except for pG-cyclic-p, all the oligonucleotides listed contain ³²P at the 5' end and a 3'-hydroxyl group. The pattern obtained is almost identical to that obtained by Piper and Clark⁸, except for the following: (1) the spot corresponding to pApGp in their fingerprint is present as pApG, (2) the spot corresponding to C-U-A-C-C-A_{OH} (ref. 8) is present in our fingerprints as pC-U-A-C-C-A_{OH}, and (3) Gp or G-cyclic-p is absent in our fingerprints. All of these differences arise because the *in vitro* labelling procedure involves removal of phosphomonoester groups present at either the 5' and/or the 3' end and their substitution by a ³²P at the 5' end. The yield of m⁷Gdm⁵CG has been consistently low (maximum obtained, 30%) in our experiments. This may be due to inefficient phosphorylation of the 5'-hydroxyl group in an oligonucleotide rich in modified nucleosides. In more recent fingerprints (ref. 5, see also Fig. 2), it has been possible to eliminate completely the spot due to ATP, which is present in this fingerprint. B surrounded by dots, blue dye marker.

With the work of Piper and Clark⁸, the most striking result from our work is the observation that the cytoplasmic initiator tRNAs from three mammalian sources, those of mouse P3 myeloma cells, rabbit liver and sheep mammary gland, are identical. It would be interesting to determine whether these sequences have diverged further during evolution or whether they were conserved at an earlier stage of evolution, and studies along these lines are now in progress.

The total nucleotide sequence of four different methionine tRNAs which are aminoacylated by *E. coli* methionyl-tRNA synthetase with about the same K_m and V_{max} are now known. These include *E. coli* tRNA_m^{Met} (ref. 13), *E. coli* tRNA_r^{Met} (ref. 14) and yeast¹⁵ and mammalian cytoplasmic initiator tRNAs⁷. Figure 3 shows a composite structure of these tRNAs. Except for the sequence of the anticodon loop, there is no extensive homology between these tRNAs. If the recognition of these tRNAs by *E. coli* methionyl-tRNA synthetase involves interactions with several different regions of the tRNA¹⁶ and of specific nucleotides within these regions¹⁷, the composite structure (Fig. 3) defines the maximum number of nucleotides which could be involved in this specific process. It is expected that sequence analyses on other methionine tRNAs which are recognised efficiently by *E. coli* methionyl-tRNA synthetase could narrow the extent of homology between these tRNAs still further¹⁸ and provide the basis for a systematic approach to studying the problem of specific recognition between methionine tRNAs and *E. coli* methionyl-tRNA synthetase^{19,20}

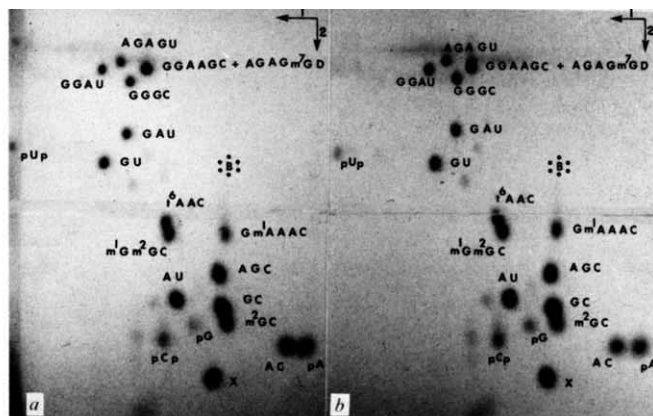


Fig. 2 Autoradiographs of 5'-³²P-labelled oligonucleotides obtained from pancreatic RNase digests of rabbit liver (a) and sheep mammary gland (b) cytoplasmic initiator tRNAs⁵. The pattern is similar to that obtained by Piper and Clark⁸ except for differences expected on the basis of the *in vitro* labelling procedure used (see legend to Fig. 1). The relative positions of t⁶AAC and m⁷Gm²GC in this fingerprint are reversed with respect to the fingerprint obtained by Piper and Clark⁸. This could be due either to minor differences between the nature of t⁶A or to the presence of phosphomonoesters at the 5' end of oligonucleotides on our fingerprints compared with 3'-phosphates in the oligonucleotides on the fingerprints obtained by Piper and Clark⁸. The radioactive spot X has been found in variable amounts in all of our fingerprints of pancreatic RNase digests of tRNAs—including non-initiator tRNAs. This probably arises from degradation of ATP by contaminating enzyme(s) in pancreatic RNase. Variable amounts of pA and pG have consistently been observed in fingerprints of pancreatic RNase digests of tRNA. We are now examining this phenomenon with regard to the possibility of traces of exonuclease contamination in the enzymes used. B surrounded by dots, blue dye marker.

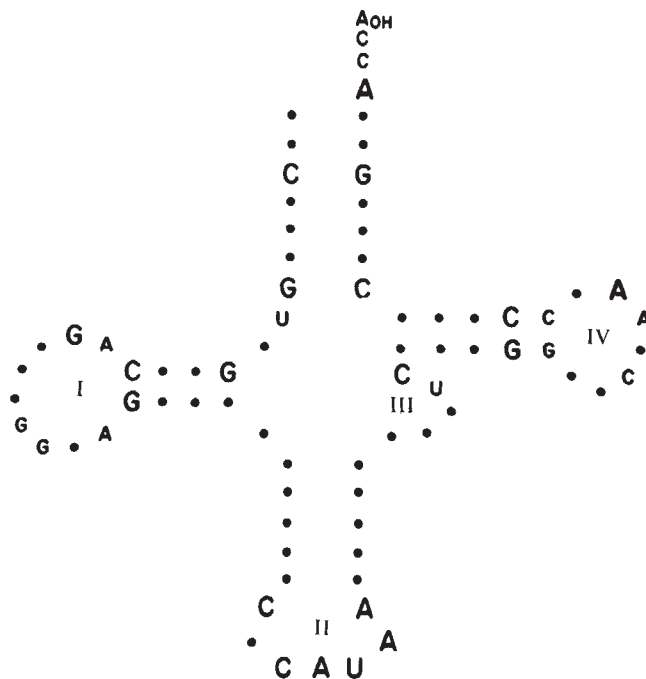


FIG. 3 Composite structure of methionine tRNAs. Nucleotides not common to these tRNAs are indicated by a dot. Nucleotides found in the same position in all tRNAs are indicated by small letter. Those uniquely common to the six methionine tRNAs are indicated by large letter.

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Cainozoic lacustrine stromatolites from hominid-bearing sediments East of Lake Rudolf, Kenya

Gary D. Johnson

Department of Earth Sciences, Dartmouth College, Hanover, New Hampshire 03755

A new occurrence of algal stromatolites from Plio-Pleistocene lacustrine littoral sediments in the Eastern Rift, Kenya is described. These biolithites provide criteria for the interpretation of the environment of deposition of many of the hominids so far collected in the East Rudolf area.

THE documented stratigraphic history of the Lake Rudolf area, Kenya, now extends back 4.5 m.y. BP¹⁻¹¹. This record of terrestrial vertebrate evolution, sedimentary lithosomal variation and volcanic events during the Plio-Pleistocene now provides the chronology for the many hominid (*Australopithecus* and *Homo*, sensu Leakey⁶⁻⁹) remains recovered from the sediments of the Koobi Fora Formation in the East Rudolf Basin.

Events of lacustrine fluctuation are important to the chronology of much of the East Rudolf sedimentary terrane and characterise the Pliocene, Pleistocene and Holocene of the area^{5,12,13}. The Lake Rudolph Basin, not always a closed basin evaporating pan, has had periodic connection with the Nile through an outlet (Sanderson's Gulf) north-west of the limits of the present lake¹². As a result of these fluctuations, complex, interdigitated lithosomes of fluvial, deltaic and littoral lacustrine character are found throughout the area of sediment outcrop.

Detailed mapping of some of the hominid-yielding localities has demonstrated habitat association with nearby lacustrine strand environments¹⁴. Many of the sites are in turn overlain by transgressive lacustrine lithosomes. Characteristic of many of the basal facies of these transgressive lithosomes are extensive algal biolithites or stromatolites.

Previous investigations

Non-skeletal, organic sedimentary structures (stromatolites) produced by the mechanical accretion by filamentous algae of calcium carbonate, clay and other particles are quite

common in the Plio-Pleistocene stratigraphic sequence of East Rudolf². These structures usually occur as laminated muddy carbonates in which the cellular fabric of the original algae is not preserved.

The usefulness of shape as an environmental indicator has been discussed¹⁵⁻²³. Three geometric forms of stromatolitic growth—mats, hemispheroids and spheroids—seem to be dominant. The environmental significance of each of these is so far unresolved, but seems to reflect various aspects of turbulence, turbidity, and/or desiccation in the littoral or supralittoral.

Various investigations of both ancient and modern lacustrine stromatolites²⁴⁻³⁰ recognise the importance of turbulence in controlling growth form. Morphological zones in Recent stromatolites of the Great Salt Lake replicate and often enhance underlying substrate configuration²⁵. Subsequent distribution and shape of individual stromatolites are controlled by erosional channel geometry developed perpendicular to the lake shore. Typical concentric growth is controlled by better aerated conditions on the leading edge of the colony. Grove and Goudie^{15,16} reported well-developed algal biolithites from various strand levels of Lake Stephanie, Ethiopia. Their observations on growth form, although preliminary, are very similar to the East Rudolf stromatolites described here.

East Rudolf algal biolithites

The sediments of the Koobi Fora Formation, Guomde Formation and Galana Boi beds have a lacustrine record which marks ancestral Lake Rudolf fluctuations. Typical proximal or near-shore to distal stromatolitic facies can be differentiated. Deep water stromatolites may exist but such examples are not abundant in Rudolf sediments.

I have mapped the East Rudolf collecting area 103 (Fig. 1, southern hatched area) using several well-developed stromatolites, as markers of lacustrine transgression, to delineate transgressive/regressive stratigraphic cycles. The stromato-

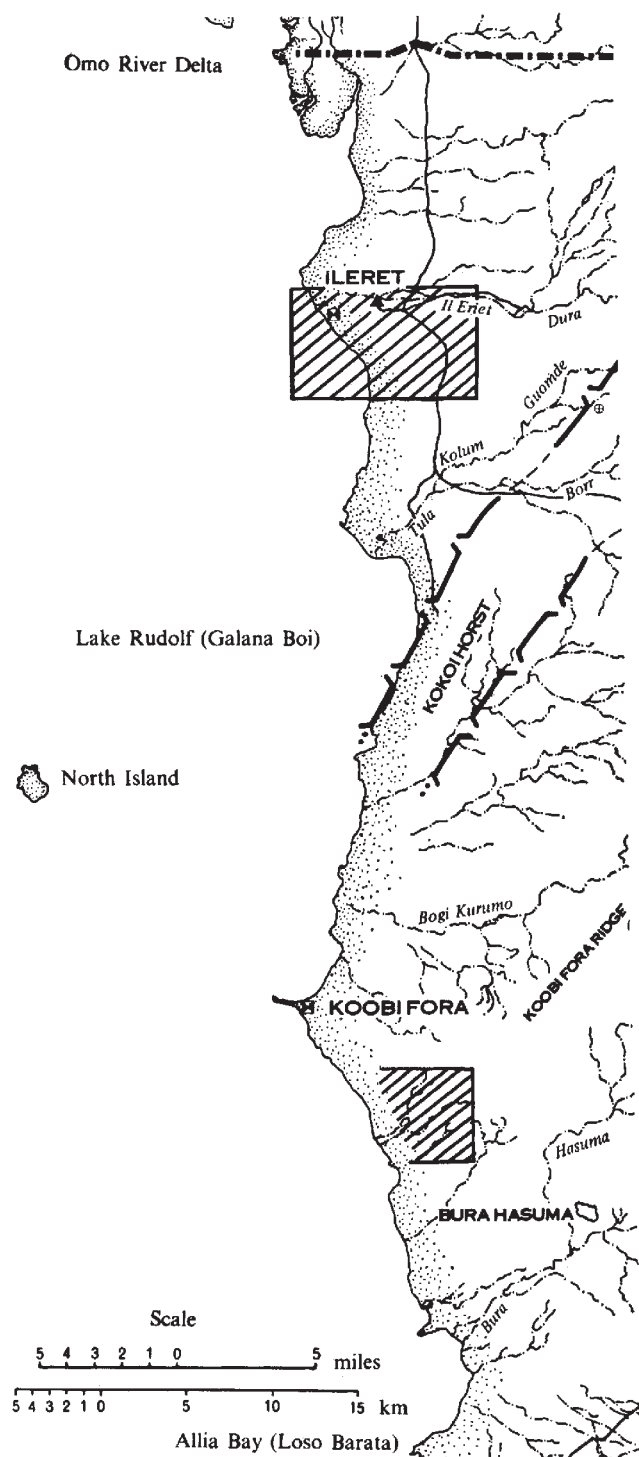


FIG. 1 Outline map of East Rudolf area showing localities of stromatolite development discussed in text.

lites which initiated these fluvial lacustrine intervals exhibit the most varied morphology and can be used to illustrate facies variability. Varied morphology gives rise to oncolites or concentrically stacked (laminated) spheroids (type SS-C; terminology after Logan, Rezek, and Ginsburg, ref. 17) and domes or vertically stacked (laminated) hemispheroids (type SH-V) to complexly compound forms (Figs 2 and 3). Analysis of all growth forms suggests open, solitary structures in more distal environments, with coalesced compound structures in more proximal environments. Several distinct facies can be recognised from the Upper Member of the Koobi Fora Formation:

Facies A. Polygonally desiccated algal mat containing a profuse gastropod epifauna (*Bellamya*). Individual polygons of thicknesses up to 3 cm have been noted. Polygonal diameters range from 10–15 cm. Throughout area 103, this facies is quite common and grades laterally into facies B (see Fig. 3d.)

Facies B. Concentric, smooth and nearly flat algal stromatolitic layers or mats. These are quite common in the central portion of area 103, and suggest fairly continuous, laterally extensive low energy environments of the shallow water lacustrine littoral. Epifauna is scarce but associated vertebrate debris derived from local distributary sources and beach strand concentrations seem to predominate. In this facies, the stromatolites have resulted from lateral and vertical accretion over an irregular substrate which may include disarticulated allochthonous bone material and/or other allochthonous clastics (Fig. 3c).

Facies C. Discontinuous algal mats. These seem to be developed in fairly restricted shallow littoral environments and exhibit a variety of growth forms ranging from isolated

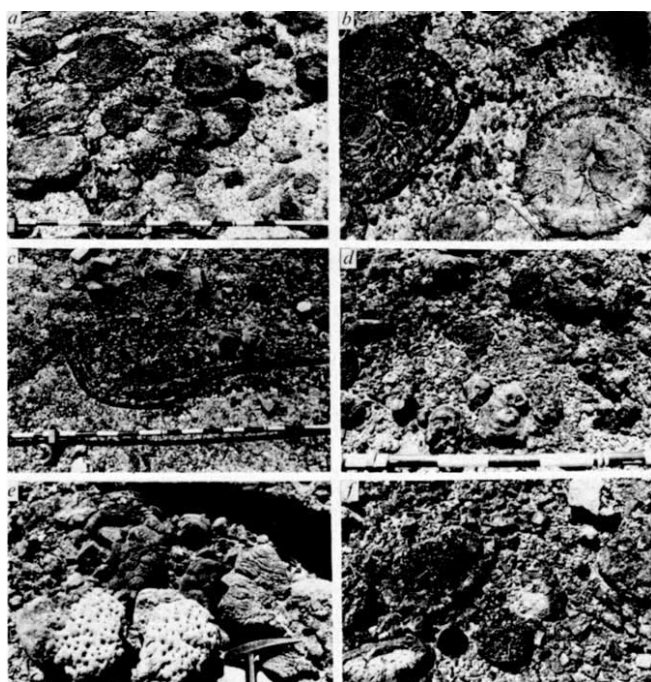


FIG. 2 Field occurrence of algal stromatolites from the Koobi Fora Formation, East Rudolf Basin, Kenya. Length of divisions on Jacob's staff, 10 cm. *a*, Coalescent vertically stacked hemispheroids. Occur as discrete stacked stromatolites passing upwards into compounded forms. Type SH-V/LLH-C of Logan *et al.* (ref. 17) modified by vertical coalescence; hence, type SH-V→LLH-C. Typical of distal (deeper lacustrine) portion of stromatolite facies in area 103. *b*, Close-up of two type SH-V→LLH-C stromatolites. Same view as *a*. *c*, Laterally coalesced stromatolite on edge of channel developed in stromatolite facies. Channel is perpendicular to the lacustrine strand. Note well developed leading edge of growth towards the well aerated channel axis. Stromatolites of the interchannel area are well developed type SH-V→LLH-C forms, all in natural growth position. Random stromatolites of type SH-V/LLH-C within the channels are commonly disturbed, and no longer in growth position. See below. *d*, Type SH-V, constant and variable basal radius stromatolites within channel areas. Note overturned domes; as many as 50% being disturbed. Channel width varies from 1–4 m across. Note relatively small initial growth surface preserved on some of the overturned forms. See *f*. *e*, Pitted type SH-V/LLH-C stromatolites from intra-channel area, area 103. Similar to pitted *Chlorellopsis* biolithites figured by Bradley (ref. 24, his plates 37 and 38). *f*, Overturned type SH-V/LLH-C stromatolites within channel area. Nuclear surface for stromatolite in lower left corner was shell of the bivalve *Mutela* sp. See *d*.

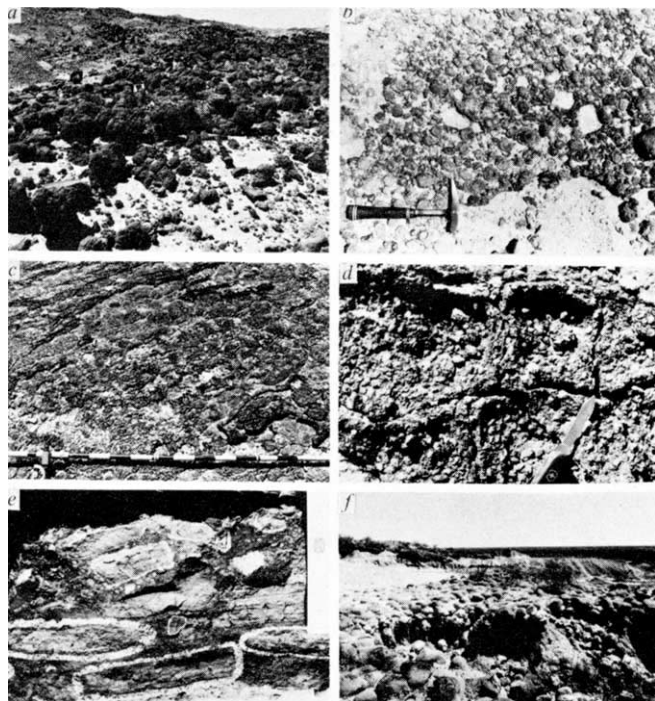


FIG. 3 Field occurrence of algal stromatolites from the Koobi Fora Formation and Galana Boi beds, East Rudolf Basin, Kenya. Length of divisions on Jacob's staff, 10 cm. *a*, Type SH-V stromatolites exhumed on extensive dip slope in central portion of area 103. Constitutes distal, turbulence-controlled facies of transgressive lacustrine lithosome. Overlying unit consists of an arenaceous mudstone which is easily winnowed from the intercolumnal areas. Stromatolites exhibit least amount of microstructure of any facies; composed of poorly sorted feldspatholithic sandstone. *b*, Type SS-C stromatolites (oncolites), area 103. Occur as extensive deposit in distal littoral facies. Nuclei are usually lithic clasts or bivalve (*Caelatura*) shells. *c*, Overview of typical stromatolitic biosome development showing general elongation of compound growth forms. Proximal, more coalesced forms to the right, out of picture. Distal, less coalesced forms to the left. Lineations are perpendicular to ancient shoreline. *d*, Desiccated algal mat facies. Profuse gastropod (*Bellamya*) epifauna occurs in this supralittoral facies. *e*, Mudstone clasts encased by stromatolitic carbonate. Note that some clasts are not encrusted evidencing mixed transport and residence time for these allochthonous shingles. *f*, Type SS-C stromatolites of the Galana Boi beds, Ileret. Largest forms may be 0.5 m in diameter. Unit grades laterally in size and nuclear material. Bench in distant background is limit of algal facies. *Etheria*, *Caelatura* are common nucleating bivalves. *Etheria*, the common river oyster, is the nucleus for most of the large forms in the foreground.

columnar structures to vertically stacked but laterally coalesced 'typical' stromatolitic structures. Logan³² considered columnar growth a response to the upward accretion of stromatolitic laminae by increment entrapment of detrital material by filamentous algae producing a vertical stacking of hemispheroidal laminae (type SH-V). Numerous evidence from studies of marine intertidal stromatolites suggests that columnar growth may not be related to random isolated centres of stromatolitic development, but rather disarticulation of a once continuous algal mat by mechanical scouring of the substrate^{21,32,33}. In this context, the large stromatolitic heads of area 103 which occur as irregular and laterally disarticulated, one-time cohesive algal surfaces have subsequently undergone coalescent algal growth.

Facies D. Compound spheroidal and hemispheroidal stromatolites. These are generally laterally extensive, occurring in distal environmental facies throughout most of the Koobi Fora Formation. Surfaces upon which stromatolites nucleate are almost always lacustrine molluscs or lithic fragments (Fig. 2a, b).

Facies E. Concentrically stacked spheroidal stromatolites (oncolites). Throughout several intervals in area 103, and north at Ileret (Fig. 1, northern hatched area) type SS spheroidal stromatolites (terminology from Logan, Rezak and Ginsburg, ref. 17) are found. These consist of stromatolitic coatings on the surface of shell debris, disarticulated vertebrate debris, and/or lithic fragments. Encrustations of less than 1 mm to complete spherical stromatolites 0.5 m in diameter are present (Fig. 3g, f).

Significance

Form and distribution of algal stromatolites in the East Rudolf terrane are significantly controlled by sediment input and subsequent reworking of the littoral lacustrine environment. As is the case with modern stromatolites of the shallow littoral of the Great Salt Lake²⁵, most stromatolites of East Rudolf seem to have responded to conditions of turbulence associated with erosion channels developed perpendicular to the ancestral Lake Rudolf strand. The regional variation, however, of the stromatolite-bearing strata is not known at present from the East Rudolf area. Mapping of microfacies variability is continuing because the lithosomal variation in East Rudolf sediments provide a wealth of palaeoenvironmental data. Detailed reconstruction of the East Rudolf palaeogeography for the interval represented by the Upper Member of the Koobi Fora Formation will be possible on the basis of these unique biogenic facies.

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Accumulation of fossil CO₂ in the atmosphere and in the sea

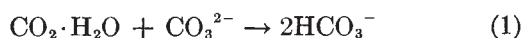
M. Whitfield

The Laboratory, Citadel Hill, Plymouth PL1 2PB

An analysis from the chemist's point of view of the uptake of industrial produced carbon dioxide by the oceans reveals that serious environmental effects are not likely in the foreseeable future.

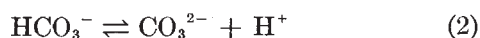
In recent months the threat posed by the accumulation of fossil CO₂ in the atmosphere has been given a new twist by the suggestion that, as a consequence, the surface layers of the ocean may become undersaturated with respect to calcium carbonate by the turn of the century¹. Similar conclusions have been reached by other workers²⁻⁵ although they did not comment on the possibly catastrophic biological consequences. Since the same predictions, with slightly differing time scales, are made using a number of mixing models^{1,2,4}. I will not consider the relative merits of the various global treatments but will dwell instead on more mundane chemical matters.

Basically all the treatments¹⁻⁵ assume that the injection of fossil CO₂ into the ocean will remove carbonate ions according to the reaction



According to Broecker *et al.*⁴ the sea will become undersaturated with respect to calcite when the carbonate content of the well mixed surface layers of the ocean has been reduced to 3.5×10^{-5} mol l⁻¹. Depending on the mixing model used this could occur well into the next century⁴, by the year 2008 (ref. 1) or even earlier^{2,3}. It is instructive to look at the chemistry of the seawater when this fateful day arrives.

A widely accepted mean recipe for seawater⁶ gives the total concentrations of the carbonate $[\text{CO}_3^{2-}]_{\text{T}}$ and bicarbonate $[\text{HCO}_3^-]_{\text{T}}$ ions in seawater as 2.69×10^{-4} mol l⁻¹ and 2.38×10^{-3} mol l⁻¹ respectively in the early 1960s. These concentrations are related by the well known equation



for which the stoichiometric constant K^*_{20} may be defined as

$$\log K^*_{20} = \log [\text{CO}_3^{2-}]_{\text{T}} / [\text{HCO}_3^-]_{\text{T}} - \text{pH} \quad (3)$$

Hansson⁷ has measured values of K^*_{20} on the seawater ionic medium scale. Using his value at 25° C and 35‰ salinity equation (3) becomes

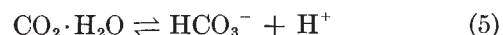
$$\text{pH} = \log [\text{CO}_3^{2-}]_{\text{T}} / [\text{HCO}_3^-]_{\text{T}} + 8.947 \quad (4)$$

giving a pH of 8 for our initial seawater. The pH values here

are also defined on the seawater ionic medium scale⁸. To reach the saturation level for calcite 2.34×10^{-4} mol l⁻¹ of carbonate ions must be removed, resulting in the formation of 4.68×10^{-4} mol l⁻¹ of bicarbonate according to reaction (1). In the saturated seawater then

$$\text{pH} = \log (3.5 \times 10^{-5} / 2.85 \times 10^{-3}) + 8.947 = 7.04$$

suggesting even more fundamental biological implications. The carbon dioxide system in seawater is, however, constricted by a further restraint, that implied by the familiar equation



for which

$$\log K^*_{10} = \log [\text{HCO}_3^-]_{\text{T}} - \log [\text{CO}_2 \cdot \text{H}_2\text{O}] - \text{pH} \quad (6)$$

Again using Hansson's⁷ value for K^*_{10} at 25° C and 35‰ salinity and the final value of 2.85×10^{-3} mol l⁻¹ for $[\text{HCO}_3^-]_{\text{T}}$ we find that

$$\log [\text{CO}_2 \cdot \text{H}_2\text{O}] = 5.857 - 2.545 - 7.04 = -3.728$$

According to Murray and Riley⁹ $[\text{CO}_2 \cdot \text{H}_2\text{O}]$ is related to the partial pressure of carbon dioxide above the seawater (P_{CO_2}) at 25° C and 35‰ salinity by the equation,

$$[\text{CO}_2 \cdot \text{H}_2\text{O}] / P_{\text{CO}_2} = 289 \times 10^{-4} \text{ mol l}^{-1} \text{ atm}^{-1} \quad (7)$$

so that the partial pressure of carbon dioxide above our saturated seawater is 6.47×10^{-3} atm which is nearly twenty times the present value (3.33×10^{-4} atm) and an order of magnitude greater than the predicted partial pressure at the time saturation is achieved. Clearly something is wrong with the chemical hypothesis.

The error apparently arises because equation (1) is treated as a one way reaction rather than as an equilibrium so that it is assumed that every molecule of CO₂ taken in consumes a molecule of carbonate. If this were so no carbonate would exist in the oceans even now and all of the atmospheric carbon dioxide would have been used up long ago to give an oceanic sodium bicarbonate solution. The equilibrium equation can be formulated by subtracting equation (2) from equation (5). To understand the effects of fossil CO₂ on the oceanic mixed layer we must assess the contributions of the various components to the state of balance of this equilibrium.

The mixing models¹⁻⁵ themselves apply three constraints on the oceanic carbon dioxide system that must be considered

in a chemical treatment. The concentration of carbon dioxide in the atmosphere at any point in time is fixed by the forward and backward projections of the current accumulation rates. The concentration of fossil carbon dioxide accumulated in the mixed layer of the oceans (and hence its total carbon dioxide content $C_T = [\text{CO}_2 \cdot \text{H}_2\text{O}] + [\text{HCO}_3^-]_T + [\text{CO}_3^{2-}]_T$) is also fixed at a level that depends on the assumptions made by the mixing model. Finally the carbon dioxide system in the mixed layer must be treated as an open system with free exchange of unhydrated CO_2 across the air-sea interface. I shall first make some rough predictions of the total concentrations of carbon dioxide in the atmosphere and in the mixed layer of the ocean at ten year intervals and will then apply these constraints to the oceanic carbon dioxide system.

The annual injection rate of carbon dioxide into the atmosphere has been calculated by Machta¹⁰ and rather more pessimistic figures, on a decade basis, are given by Broecker *et al.*⁴ Mixing models developed from these figures^{1-4,10} indicate that only 60% of the carbon dioxide released into the atmosphere accumulates there. Most of the remaining 40% is removed by the oceans^{4,10}. Assuming, along with Broecker *et al.*⁴, that this partition will remain fairly constant if the current increase in production of 4.5% yr^{-1} continues, then values can be calculated for the atmospheric concentration of carbon dioxide (mol l^{-1}) at the end of each decade. I will take 1958 as my starting point using a mean world value¹⁰ of $1.397 \times 10^{-5} \text{ mol l}^{-1}$ (313 p.p.m.) for the atmospheric concentration of carbon dioxide. For Machta's data¹⁰ the total injection of carbon dioxide since 1958 is given by

$$\begin{aligned}\Delta\text{CO}_2 &= (1.1/w_c V_a) \Sigma_i \delta_{i(\text{M})} \\ &= 2.333 \times 10^{-7} \Sigma_i \delta_{i(\text{M})}\end{aligned}\quad (8)$$

where the annual increments ($\delta_{i(\text{M})}$) are given by Machta¹⁰ in units of $10^{16} \text{ g carbon per year}$, w_c is the atomic weight of carbon and V_a is the volume of the atmosphere¹ ($3.93 \times 10^{21} \text{ l}$). The factor 1.1 allows for Machta's corrections to his tabulated values¹⁰. The actual accumulation of carbon dioxide

in the atmosphere since 1958 is taken as $0.6 \Delta\text{CO}_2$. Broecker *et al.*⁴ give their data in units of 10^{16} g CO_2 per decade so that

$$\begin{aligned}\Delta\text{CO}_2 &= (w_{\text{CO}_2} V_a)^{-1} \Sigma_i \delta_{i(\text{B})} \\ &= 5.783 \times 10^{-8} \Sigma_i \delta_{i(\text{B})}\end{aligned}\quad (9)$$

The resulting change in C_T in the mixed layer of the oceans is given by the equation

$$\Delta C_T = (10^3 V_a / 0.9 A d) y \Delta\text{CO}_2 \quad (10)$$

where y is the fraction of the fossil CO_2 injection assimilated into the mixed layer, A is the surface area of the oceans¹¹ ($3.61 \times 10^{14} \text{ m}^2$) and d is the mean depth of the mixed layer⁴ (70 m). The mixed layer is only formed over 90% of the ocean surface^{1,4}. Substituting in the numerical values we find,

$$C_T = 2.650 \times 10^{-3} + 4.031 \times 10^{-5} y \Sigma_i \delta_{i(\text{M})} \quad (11)$$

or

$$C_T = 2.650 \times 10^{-3} + 9.990 \times 10^{-6} y \Sigma_i \delta_{i(\text{B})} \quad (12)$$

According to the model of Broecker *et al.*⁴ only 5% of the total fossil CO_2 remains in the mixed layer so that $y = 0.05$. Machta¹⁰, on the other hand, suggests that $y = 0.25$ and this is more in line with Fairhall's model¹. The most pessimistic

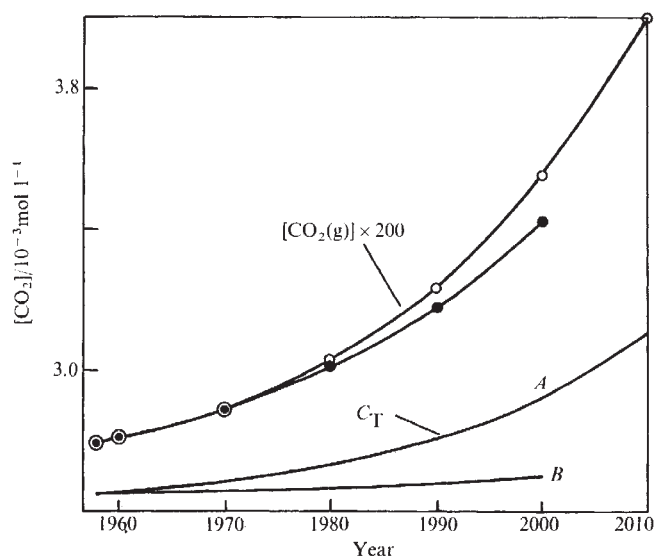


FIG. 1 Cumulative concentrations of carbon dioxide in the atmosphere (upper set) and in the oceanic mixed layer (lower set). O, Using Broecker's injection figures⁴. ●, using Machta's injection figures¹⁰. A uses Broecker's⁴ injection figures and $y = 0.25$ (refs 1, 10) (equation (12)) and B uses Machta's injection figures¹⁰ and $y = 0.05$ (ref. 4) (equation (11)). These calculations represent respectively the highest and lowest predictions for the accumulation of CO_2 in the mixed layer.

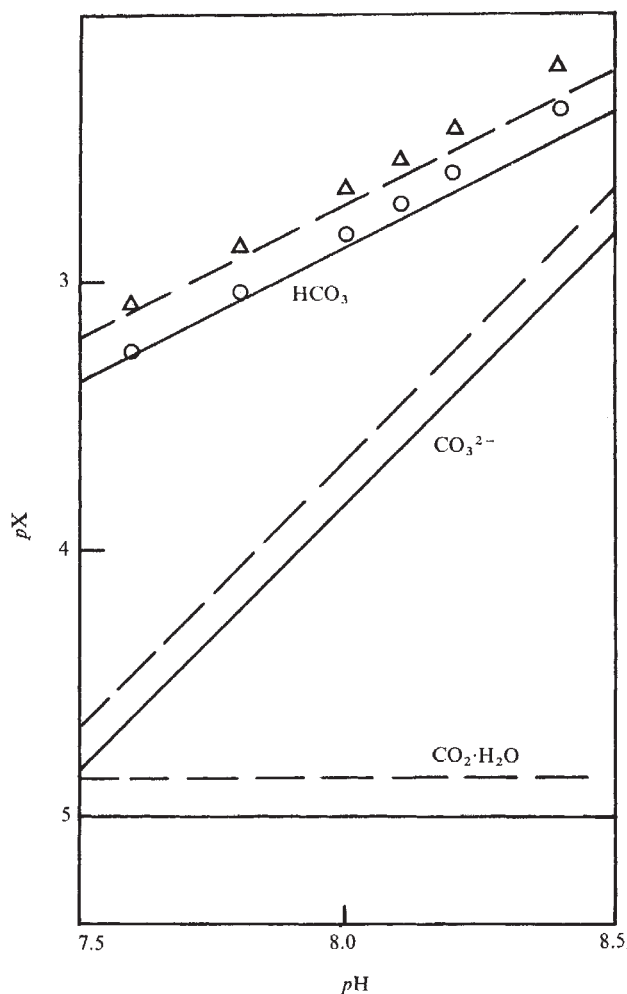


FIG. 2 Concentrations of the various components of the carbon dioxide system in the mixed layer plotted on a logarithmic basis against pH (equations (13) to (15)). —, Concentrations in 1958; — — —, concentrations in 2010. Calculated total concentrations are also indicated (Δ , 2010; O, 1958).

estimates of the accumulation of fossil CO_2 in the mixed layer then are obtained by using $y = 0.25$ in equation (12) (Fig. 1, curve A). By this estimate C_T will have reached $3.103 \times 10^{-3} \text{ mol l}^{-1}$ and $[\text{CO}_2(\text{g})] 2.027 \times 10^{-5} \text{ mol l}^{-1}$ by the year 2010 (Fig. 1, curve A). This represents a more rapid

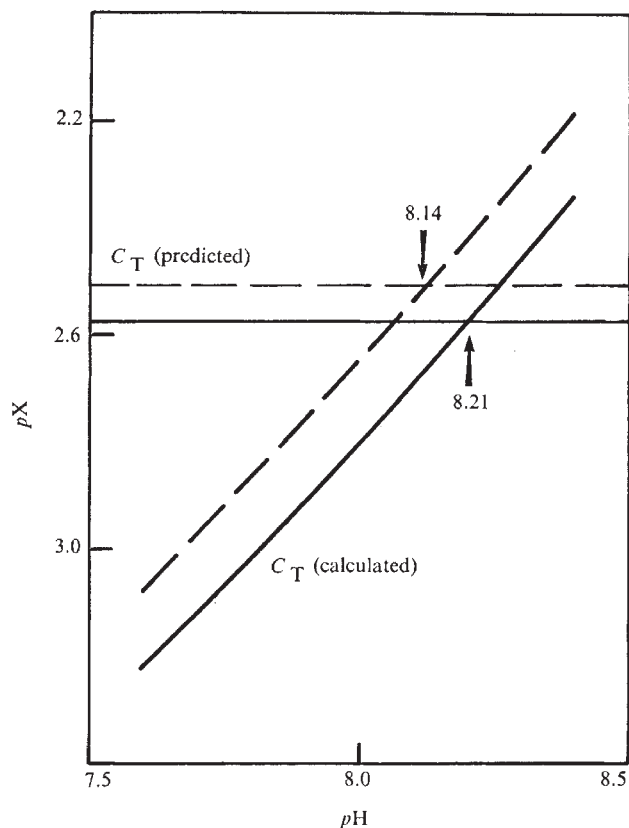


FIG. 3 Estimation of the equilibrium pH of seawater in the mixed layer in 1958, ——— and 2010 (— — —). The pH values (arrowed) are given by the points where the curves showing values of C_T calculated from equations (13) to (14) cross the horizontal lines representing the values of C_T predicted from equation (12) with $y = 0.25$.

accumulation of fossil CO_2 in the mixed layer than was assumed by Fairhall¹. What will be the state of the oceanic carbon dioxide system at this time?

To answer this question we must be able to estimate the total concentrations of carbon dioxide and of carbonate and bicarbonate ions in the mixed layer. These parameters together will fix the oceanic pH. The concentration of carbon dioxide in the mixed layer is related directly to the concentration of carbon dioxide in the atmosphere by equation (7). Since $[\text{CO}_2(\text{g})]$ is fixed by the mixing model (Fig. 1, open circles) we can treat the oceanic system at any point in time as an open system¹² with the concentration of unionised carbon dioxide (mol l^{-1}) fixed by the equation

$$[\text{CO}_2 \cdot \text{H}_2\text{O}] = 0.647[\text{CO}_2(\text{g})] \quad (13)$$

at 25°C and total pressure 1 atm. The concentration of bicarbonate ions can be obtained by re-arranging equation (6) to give¹²

$$\log [\text{HCO}_3^-]_T = (\log [\text{CO}_2 \cdot \text{H}_2\text{O}] + \log K^*_{1c}) + \text{pH} \quad (14)$$

Similarly by adding equations (3) and (6) and rearranging we find that

$$\log [\text{CO}_3^{2-}]_T = (\log [\text{CO}_2 \cdot \text{H}_2\text{O}] + \log K^*_{1c} + \log K^*_{2c}) + 2\text{pH} \quad (15)$$

To fix the actual concentrations of these components (and hence the pH) we must use the second constraint applied by the mixing models, the known value of C_T at any point in time. A particularly simple analysis is possible if we plot the logarithm of the concentrations of each component ($-\log X = \text{pX}$) against pH since the distribution of each species is then represented by a series of straight lines (Fig. 2). By summing these concentrations at regular pH intervals a cumulative curve showing $-\log C_T$ as a function of pH can be established (C_T (calculated), Fig. 3). The properties of the oceanic system will then be defined by the point at which this curve cuts the horizontal line corresponding to the value of C_T predicted by the mixing model (Fig. 3).

For the seawater at our arbitrary starting point in 1958 this gives $[\text{CO}_2 \cdot \text{H}_2\text{O}] = 9.05 \times 10^{-6} \text{ mol l}^{-1}$, $[\text{HCO}_3^-]_T = 2.20 \times 10^{-3} \text{ mol l}^{-1}$, and $[\text{CO}_3^{2-}]_T = 4.33 \times 10^{-4} \text{ mol l}^{-1}$ at pH of 8.24 (Fig. 2). These results differ slightly from those calculated using equation (6) since the model used there⁶ treats the ocean as a closed system. By the year 2010, according to the most pessimistic estimates, $[\text{CO}_2(\text{g})]$ will have reached $2.027 \times 10^{-5} \text{ mol l}^{-1}$ so that $[\text{CO}_2 \cdot \text{H}_2\text{O}] = 1.312 \times 10^{-5} \text{ mol l}^{-1}$. Using this value and the predicted value of C_T ($3.103 \times 10^{-3} \text{ mol l}^{-1}$) the graphic method (Figs 2 and 3, dashed lines) predicts an equilibrium seawater with a pH of 8.16 and the following concentrations; $[\text{HCO}_3^-]_T = 2.65 \times 10^{-3} \text{ mol l}^{-1}$ and $[\text{CO}_3^{2-}] = 4.36 \times 10^{-4} \text{ mol l}^{-1}$. The mixed layer is able to accommodate the fossil CO_2 injected by even the most pessimistic model without drastic changes in pH and without shifting the carbonate solubility equilibria appreciably.

The results of the calculations account, in a self-consistent manner, for the predicted atmospheric and oceanic concentrations. No account need be taken of the calcium carbonate equilibria since the mixed layer is supersaturated throughout, and is likely to remain so.

If the mixing models are correct in their predictions at C_T and $[\text{CO}_2(\text{g})]$ then a gross supersaturation at the mixed layer with $[\text{CO}_2 \cdot \text{H}_2\text{O}]$ is required to produce carbonate dissolution.

This result is reassuring since it indicates that the assimilation of carbon dioxide in the mixed layer will not in itself give rise to any biological catastrophe¹. It does, however, imply that the extra buffering mechanism for the absorption of carbon dioxide provided by the dissolution of the carbonate sediments⁴ will not come into play until the bulk of the fossil CO_2 has penetrated the deep ocean. The consequences of man's profligate use of fossil fuel will therefore be apparent in the atmosphere for some time.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Detection of formaldehyde in external galaxies

FURTHER to our observations of OH in the edge-on spiral galaxies NGC253 and NGC4945 (ref. 1), we now report the detection of H_2CO absorption in both galaxies—the first evidence of this molecule in external galaxies.

The observations were made in early November 1973 with the Parkes 64-m telescope equipped with a 6-cm cryogenic parametric amplifier and a 512-channel autocorrelator. The latter was used in the total power mode. The correlator bandwidth was 10 MHz and the effective resolution was 19.5 kHz (equivalent to 1.2 km s^{-1} at the H_2CO rest frequency of 4,829,660 MHz). The beamwidth of the telescope was 4.2 arc min.

The H_2CO spectra obtained with the telescope directed towards the continuum maxima (coincident in position with

the optical nuclei) of NGC253 and NGC4945 are shown in Figs 1 and 2 respectively. For comparison purposes OH and HI spectra obtained in similar directions are also shown; these have been discussed previously¹. The H_2CO results represent about 6 h of integration on-source; an equal amount of time was spent at reference positions. Since no narrow spectral features exceeding the noise were found, the profiles shown have been smoothed to an effective resolution of 20 km s^{-1} .

For both galaxies the H_2CO absorption profiles are easily distinguishable. Although they are reasonably similar in shape and velocity coverage to their OH counterparts, the absorption at the more positive velocities is enhanced relative to the absorption at the lower velocities.

For NGC253 the H_2CO absorption, which has a maximum antenna temperature of 0.012 K, probably occurs against the

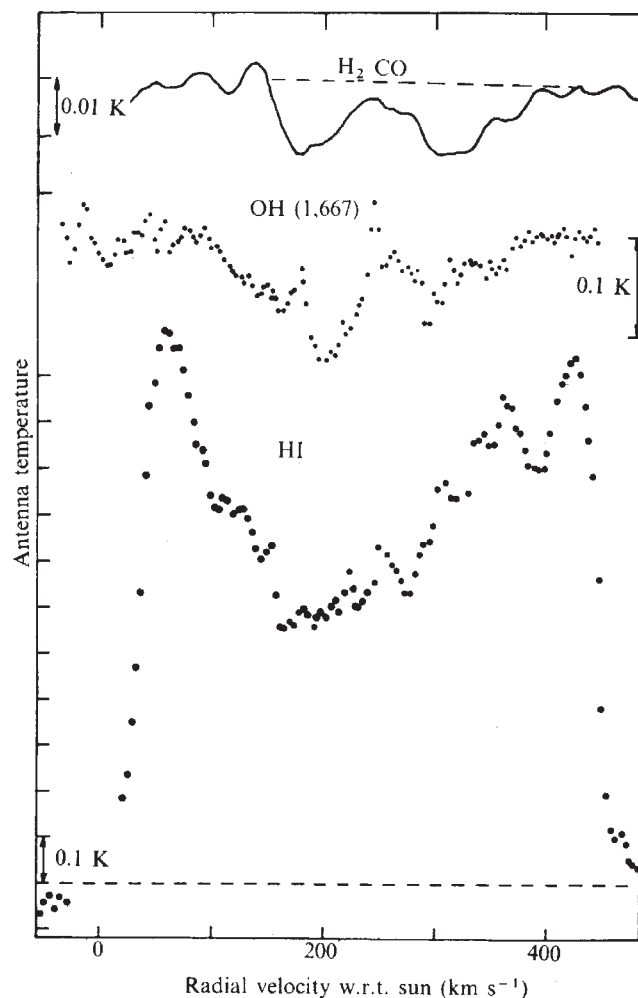


FIG. 1 Absorption profile for H_2CO in NGC253, smoothed to an effective resolution of 20 km s^{-1} in velocity. Comparison profiles for OH (1,667 MHz) and neutral hydrogen (1,420 MHz) are included.

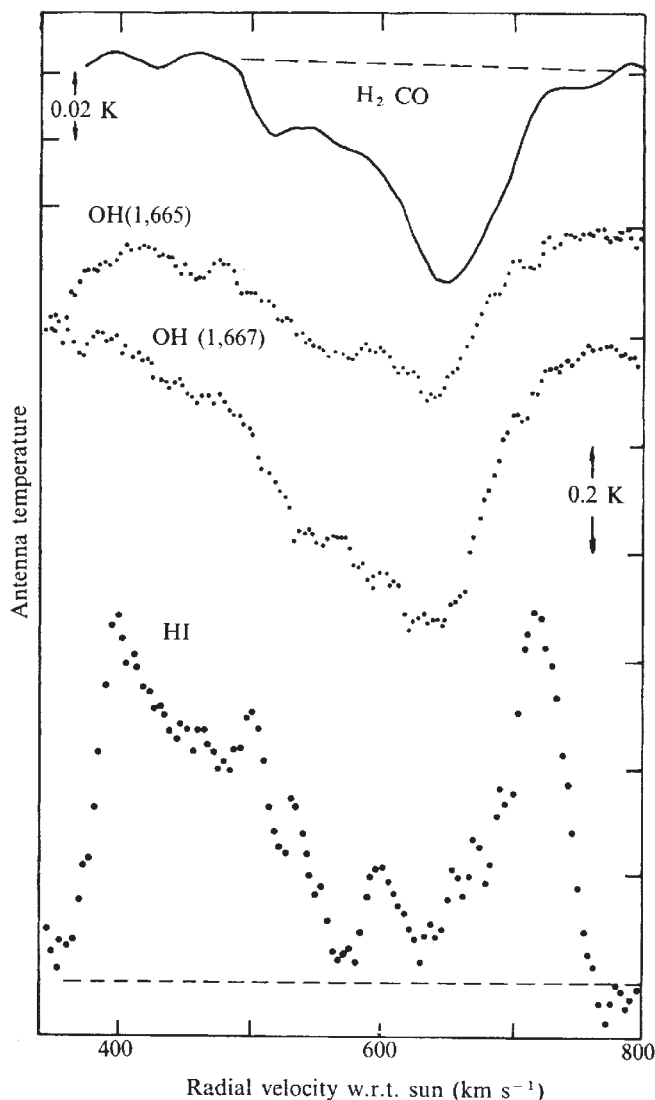


FIG. 2 Absorption profile for H_2CO in NGC4945, smoothed to an effective resolution of 20 km s^{-1} in velocity. Comparison profiles for OH (1,665 and 1,667 MHz) and neutral hydrogen (1,420 MHz) are included.

nuclear continuum component, as with the OH absorption. The maximum optical depth is about 0.02, considerably less than the corresponding value (0.07) for the OH absorption. There is no distinct feature that corresponds to the narrow-band OH emission near 250 km s^{-1} .

For NGC4945 spectra obtained at positions displaced from the nucleus by about ± 3 arc min along the major axis were consistent with the absorption of an unresolved source coincident with the nucleus. If it is assumed that about 80% of the continuum flux at 6 cm originates in the nucleus, the antenna temperature of the peak absorption (0.066 K) corresponds to an optical depth of about 0.08. For the OH absorption the maximum optical depth is 0.3 (ref. 1). Compared with the molecular absorption in the Galaxy the optical depth of the H_2CO is unusually low—our (unpublished) observations of galactic sources suggest that the opacity of H_2CO generally exceeds that of OH when the optical depth of OH is greater than about 0.15.

It is worth noting that the relative enhancement of the H_2CO absorption at the more positive velocities, mentioned earlier, occurs also in the nuclear region of the Galaxy, where the ratio {optical depth (H_2CO):optical depth (OH)} is greater for the $+40 \text{ km s}^{-1}$ absorption than for the -130 km s^{-1} . The presence of absorption at radial velocities more positive than the systemic velocity in NGC253, in NGC4945, and in the Galaxy is most readily explained in terms of material moving towards the nucleus. It may be a common feature of the nuclear regions of spiral galaxies.

F. F. GARDNER
J. B. WHITEOAK

Division of Radiophysics, CSIRO,
Sydney, Australia

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Radio structure of the 'double quasar' 4C11.50

WAMPLER *et al.*¹ have recently announced the discovery of a close pair of optical quasars associated with the radio source 4C11.50. The brighter quasar (a) has a redshift of 0.436, whereas its companion (b), 4.8 arc s away in position angle 95° , has a much larger redshift of 1.901. The statistical probability of a chance coincidence is small and this has led to speculation that, despite the discordant redshifts and the lack of any visible connection, the objects may be physically associated. Hazard *et al.*² report unpublished observations from Cambridge and Westerbork which suggest that the radio structure is double, with one component coincident with quasar a. Here, we investigate further the alignment of the radio structure, seeking in particular any evidence of a radio connection between the two optical quasars.

The observations were obtained with radio link interferometers at Jodrell Bank during the period 1973 November–December. Details of the observing frequencies and baselines are given in Table 1.

TABLE 1 Parameters of Jodrell Bank interferometers used in the observations of 4C11.50

Home station	Out station	Observing frequency (MHz)	Baseline length (wavelengths)	Minimum lobe spacing (arc s)
Mark Ia	Defford	408	172,700	1.2
Mark II	Mark III	962	76,400	2.7
Mark Ia	Mark III	1666	132,300	1.6

The variation of fringe amplitude with hour angle for each of the three baselines is shown in Fig. 1. Parameters which describe the radio structure have been assigned using a model fitting technique which assumes components with elliptical gaussian distributions of surface brightness. Details of the best fitting models are given in Table 2 and the corresponding visibility curves are shown by the solid lines in Fig. 1. Although the separation and position angle of the double structure at 408 and 962 MHz are well defined,

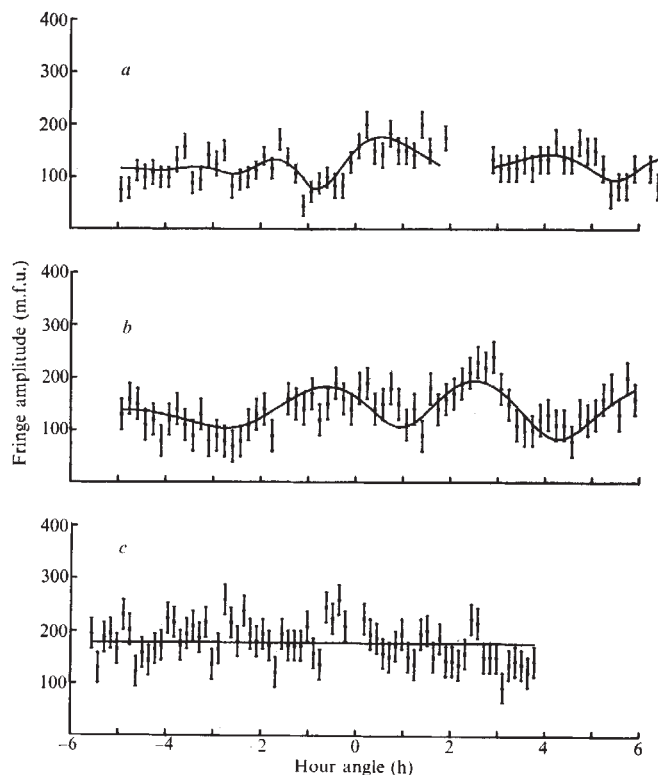


Fig. 1 Variation of fringe amplitude with hour angle at a, 408 MHz; b, 962 MHz; c, 1,666 MHz.

TABLE 2 Parameters of the best fitting models

Frequency (MHz)	Separation (arc s)	Position angle (degrees)	Component one		Component two		
			Flux density (m.f.u.)	Size (arc s)	Flux Density (m.f.u.)	Size (arc s)	Position angle of elongation (degrees)
408	14.0 ± 1.0	170 ± 2	115 ± 12	<0.3	65 ± 25	1.3 ± 0.4	65 ± 25
962	12.8 ± 1.0	168 ± 2	146 ± 15	<0.6	77 ± 35	1.5 ± 0.7	30 ± 40
1,666	—	—	179 ± 18	<0.4	—	—	—

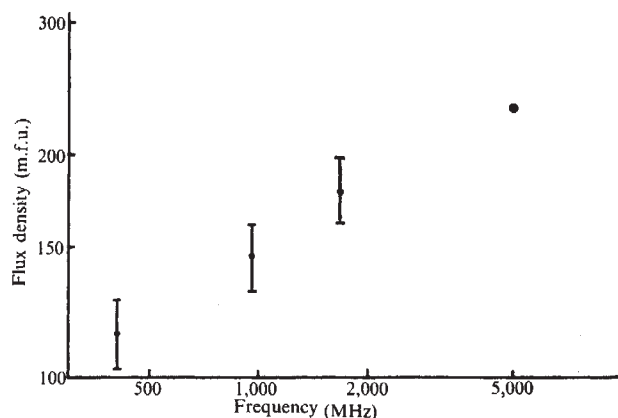


FIG. 2 Radio spectrum of the compact component coincident with 4C11.50a.

the parameters of the weaker component are more uncertain. At 1,666 MHz any variation of fringe amplitude is weak. Simple double models failed to provide a satisfactory fit to the data, which do not justify the fitting of any model more complicated than a single unresolved component.

The observations at 962 and 1,666 MHz were obtained using an improved local oscillator system developed by R. E. Spencer and R. S. Warwick (unpublished). At both frequencies the resulting phase stability over a period of several hours is sufficient to derive a position in right ascension for the more intense component. This position coincides, within our error of ± 2 arc s, with the optical position of quasar a given by Wills *et al.*³ The short term phase stability is not good enough to say whether the weaker component lies to the east or west of this position, and hence there is an ambiguity of 180° in the position angle of the double structure given in Table 2.

The stronger component is unresolved at all three frequencies with an angular diameter $\lesssim 0.5$ arc s. The derived flux densities may therefore be used to construct a spectrum. This is shown in Fig. 2, where we have also included a measurement made with the Westerbork synthesis telescope at 5 GHz (R. D. Ekers, private communication). The radio spectrum is inverted, with a spectral index α (defined in the sense that $S \propto \nu^\alpha$) of $+0.29$. This combination of a compact structure and an inverted spectrum suggests that the component may well be variable at centimetre wavelengths.

At 408 MHz the flux density accounted for by our model is only 0.18 f.u., considerably less than the zero spacing flux density of 1.8 f.u. given by Hazard *et al.*² This discrepancy indicates that extended structure of low surface brightness is present, to which our high resolution interferometers are insensitive. Synthesis observations at lower spatial frequencies are needed to define such structure. The observations at 408 and 962 MHz show, however, that the compact component associated with quasar a has a resolved companion situated about 13 arc s away in position angle 170° . At 1,666 MHz, where the resolution is comparable with that at 408 MHz, any contribution from this component is less than 30 m.f.u., which implies that it has a normal radio spectrum. The angular structure and component spectra of 4C11.50 therefore show some similarity to those of the quasar 3C273. In the case of 4C11.50 however, the secondary component is elongated in a position angle somewhat different from that of the main double structure.

At all three frequencies the observations enable us to place an upper limit of 30 m.f.u. on any radio emission from compact structure associated with quasar b. Neither the axis of the double radio source, nor the elongation of

the extended component is directed at quasar b. Thus the evidence on the radio structure of 4C11.50 presented in this paper suggests that there is no radio association between the two optical quasars.

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D. STANNARD
P. N. WILKINSON
R. S. WARWICK
R. J. DAVIS

University of Manchester,
Nuffield Radio Astronomy Laboratories,
Jodrell Bank,
Macclesfield, Cheshire

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Airborne measurement of the temperature of the cosmic microwave background at 3.3 mm

THE 'primaeval fireball' model of the Universe predicts a cosmic microwave background radiation field which has the spectral character of a blackbody source. The radiation arises from an initially very dense, very hot Universe, and has been cooled to around 2.7 K through the cosmological expansion. Previous ground-based radiometric measurements^{2–6} at wavelengths as short as 3.3 mm are consistent with a 2.7 K blackbody, but most of these points are in the Rayleigh-Jeans tail of such a blackbody spectrum.

The short wavelength direct radiometric points are shown in Fig. 1. The expected curvature of the spectrum shown in Fig. 1 should be observable even at 3.3 mm, however, and both existing measurements^{7,8} in this atmospheric window are inconsistent with a gray body or power law spectrum. They are consistent with one another and the blackbody model. Even though both experiments were performed at high altitude sites in an attempt to minimize atmospheric emission from H₂O and O₂, in no case was the atmospheric signal less than nine times the signal attributed to the background radiation. By carrying a radiometer above the tropopause, the H₂O and O₂ contribution is reduced to the point that the atmospheric emission is roughly equal to the cosmic signal.

Because the blackbody nature of this observed radiation is crucial to the validity of the big bang hypothesis and the departure of the spectrum from a power law can be checked at a wavelength of 3.3 mm, we attempted to obtain direct radiometric data free from large atmospheric effects by constructing a 3.3 mm Dicke radiometer designed specifically for mounting in a Lear Jet aircraft made available by the NASA Ames Research Center at Moffett Field, California. The instrument was carried above the tropopause on five flights in May and July of 1971; but only one flight was successful. During the course of that flight, we made 21 measurements of the background intensity at various zenith angles and report here a minimum variance weighted average of those points indicating a thermodynamic temperature of (2.48 ± 0.54) K.

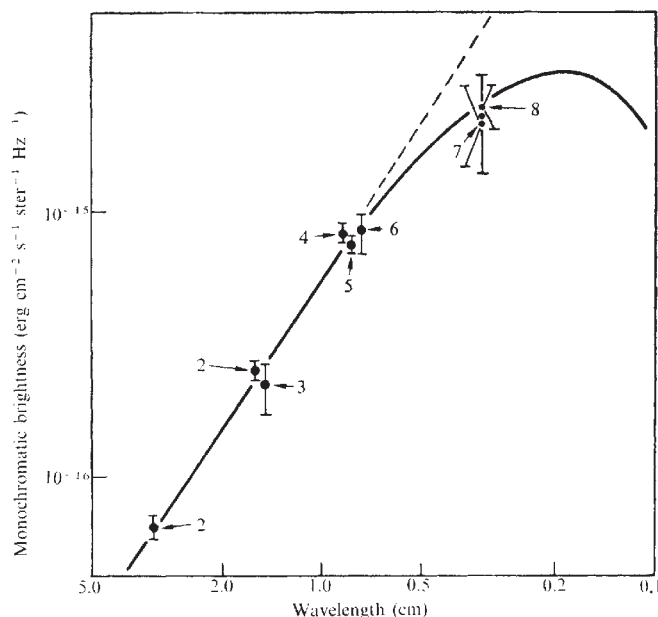


FIG. 1 Measurements of the cosmic background. Numbers indicate appropriate references. —, 2.7 K blackbody; ---, grey body.

The radiometer uses a balanced mixer with Schottky barrier diodes and a 500 to 1,000 MHz intermediate frequency. The overall noise figure of the radiometer was about 11dB, giving an r.m.s. temperature of ± 0.1 K for an integration time of 1 s.

We paid particular attention to the stability of the secondary reference standard which was used to provide a constant radiation temperature at one port of the Dicke switch. A high quality microwave termination was immersed in boiling liquid helium to provide the necessary low temperature blackbody reference source. The temperature profile along the waveguide connecting the helium load to the switch was controlled by two additional fixed points, one at the boiling point of liquid nitrogen, the other at a closely regulated temperature of 30° C. Altogether, five critical waveguide sections were electronically temperature controlled and independently monitored. In addition, the entire r.f.-i.f. system was enclosed in an insulated box within which temperature-controlled air was circulated. An additional control system was used to keep the liquid helium level on the waveguide fixed despite changes in cabin attitude or altitude.

TABLE 1 Results of airborne measurements made with the 3.3 mm radiometer

Time	Sec θ	$T_{\text{SKY}} - T_{\text{CL}}$ (K)
1356	2.28	$+0.64 \pm 0.29$
1358	1.70	-0.14 ± 0.29
1400	1.39	-0.02 ± 0.45
1401	1.70	$+0.05 \pm 0.37$
1410	1.70	-0.25 ± 0.29
1411	1.29	$+0.00 \pm 0.27$
1420	1.94	$+1.12 \pm 0.27$
1426	1.14	$+0.25 \pm 0.26$
1429	1.94	$+1.13 \pm 0.45$

With the radiometer mounted in the plane, the radiation entering the antenna, T_{SKY} , is the sum of the background radiation, T_{BG} , and atmospheric radiation, T_{ATM} . This signal is compared at 1,000 Hz with the signal from the secondary reference standard. Just before and after the flight, the radiometer is calibrated by mounting it on a special stand outside the plane and viewing the primary reference standard

with the antenna. The primary reference standard consists of a microwave absorber immersed in liquid helium; the conical taper of the antenna is continued into the dewar⁷. The signal entering the antenna during the calibration, T_{CL} , is composed of radiation from: (1) the cold absorber, T_{HE} ; (2) the metal wall of the cold-load pipe, T_{WALL} ; (3) the cold-load window, T_{WINDOW} ; and (4) reflection of radiation back into the horn by the load, T_{CLR} . The Dicke switch compares the two signals T_{CL} and T_{SKY} in turn with the signal from the secondary reference standard and if one forms the difference $T_{\text{SKY}} - T_{\text{CL}}$, the switch asymmetry and the secondary reference signal are eliminated. The various other signals are measured in independent experiments, leaving a single unknown, the background temperature, T_{BG} , which is given by

$$T_{\text{BG}} = (T_{\text{SKY}} - T_{\text{CL}}) - T_{\text{ATM}} + T_{\text{HE}} + T_{\text{WALL}} + T_{\text{WINDOW}} + T_{\text{CLR}} \quad (1)$$

By taking data with the aircraft maintaining several different constant bank angles, it was possible to obtain a fit to a simple atmospheric model. The term T_{ATM} in equation (1) is given by $T_0 \sec \theta$ is the zenith angle and $T_0 = (1.21 \pm 0.37)$ K was inferred from the fit. The last four terms in equation (1) were determined during the earlier ground-based experiment which used the same primary reference standard;³ the contributions were found to be:

$$T_{\text{WALL}} = 0.22 \pm 0.11 \text{ K}$$

$$T_{\text{WINDOW}} = 0.27 \pm 0.04 \text{ K} \quad (2)$$

$$T_{\text{CLR}} = 0.02 \pm 0.01 \text{ K}$$

$$T_{\text{HE}} = 2.40 \pm 0.01 \text{ K}$$

During our flight, 32 sky measurements were made from an altitude of 49,000 feet on July 19, 1971; the results are summarised in Table 1. Visual inspection of the radiometer chart tracing indicated a rapid drift in the output between the points taken at 1411 and those at 1420. The origin of the drift has not been determined. The remaining 11 points were deemed unreliable and are not included in our determination of T_{BG} . A weighted average of the first 21 values of T_{BG} gives

$$T_{\text{BG}} = (0.92 \pm 0.40) \text{ K} \quad (3)$$

where the error is the standard deviation in the mean (weighted by the inverse variance of $T_{\text{SKY}} - T_{\text{CL}}$ for each point). If we assume that the radiation is from a blackbody, the thermodynamic temperature which would produce this antenna temperature is

$$\tau_{\text{BG}} = \left(2.48 \pm \begin{smallmatrix} 0.50 \\ 0.54 \end{smallmatrix} \right) \text{ K at } \lambda = 3.3 \text{ mm} \quad (4)$$

The previous results at this wavelength are:

$$\tau_{\text{BG}} = \left(2.46 \pm \begin{smallmatrix} 0.40 \\ 0.44 \end{smallmatrix} \right) \text{ K (ref. 7)} \quad (5)$$

$$\tau_{\text{BG}} = (2.61 \pm 0.25) \text{ K (ref. 8).}$$

The new result is consistent with these, but most importantly, the atmospheric background subtracted from our result is only one seventh of that subtracted from the measurements.

A greybody source would produce an antenna temperature 2.7 times our result.

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PAUL E. BOYNTON

Department of Astronomy,
University of Washington,
Seattle, Washington

ROBERT A. STOKES*

Department of Physics and Astronomy,
University of Kentucky,
Lexington, Kentucky

Received September 17, revised December 5, 1973.

* Present address: Battelle Observatory Richland, Washington 99352

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Upper bound on the electric charge of a black hole

THROUGH an analysis of reversible transformations of black holes, Christodoulou and Ruffini¹ have shown that the energy of a rotating, charged black hole is given by

$$E^2 = (m_{ir} + Q^2/4m_{ir})^2 + L^2/4m_{ir} \quad (1)$$

where m_{ir} is the irreducible mass of the black hole, Q its charge and L its angular momentum. They have also shown¹ that for a given irreducible mass, the charge and angular momentum must satisfy the condition

$$L^2/4m_{ir}^2 + Q^4/16m_{ir}^4 \leq 1 \quad (2)$$

Once the equality is satisfied, no more charge or angular momentum can be added to the black hole without increasing m_{ir} .

A different expression for an upper bound on the black hole's charge can be derived by considering a loss of charge by pair production. If the electric field of the black hole creates an electron-positron pair outside the event horizon, one of these particles can enter the black hole and the other can travel to infinity. This process will occur only if the decrease in total energy of the black hole for a unit change in charge is sufficient to produce a free electron (or positron). The following discussion is limited to negatively charged black holes so that the ingoing particles are positrons. For positively charged black holes, one interchanges electrons and positrons.

In order to compute the energy of the ejected electron, we differentiate equation 1 to obtain

$$\Delta E = (1/E)(m_{ir} + Q^2/4m_{ir})(Q/2m_{ir}) \Delta Q + \Delta L \partial E / \partial L + \partial E / \partial m_{ir} \Delta m_{ir} \quad (3)$$

ΔE is the change in the energy of the black hole and ΔQ the change in its charge. For $\Delta L = 0$ and $\Delta Q = Q_e$, the electron charge, equation (3) yields

$$f m_e = (1/E)(m_{ir} + Q^2/4m_{ir}) Q Q_e / 2m_{ir} \quad (4)$$

where m_e is the electron charge and f gives a measure of the kinetic energy of the ejected electron and any increase in m_{ir} , associated with the process. The latter effect, an increase in m_{ir} , requires some explanation. It is possible that some of the energy obtained by decreasing the electric charge increases the irreducible mass; however, equations (3) and (4) assume that m_{ir} is constant. For $\Delta m_{ir} > 0$, the energy change calculated with equation (3) must exceed the final energy of the ejected particle in order to increase m_{ir} . The energy change is thus formally treated in two steps, a change in charge alone followed by a change in m_{ir} alone. The energy associated with the change in m_{ir} is included in the analysis by properly choosing f . Equation (4) will of course be useful only if we can obtain a minimum value of f for which particle ejection is possible because this value of f will determine the upper limit on the charge of the black hole.

If the ejection process can just occur, the electron will reach infinity with zero kinetic energy. Thus, a minimum value of f larger than 1 corresponds to an increase of m_{ir} . Since we can certainly create the positron with a minimum total energy (kinetic + gravitational) of m_e , the minimum value of f is less than 2. The $f = 2$ case corresponds to the creation of a free electron and free positron, each with zero kinetic energy at infinity, one of which falls back into the black hole. It is quite reasonable, however, to believe that $f = 1$. This latter statement follows from Christodoulou and Ruffini's result¹ (based on a mechanism proposed by Penrose²) which shows that it is possible to change the charge and angular momentum of a black hole such that m_{ir} does not change. An appropriate value of the positron's energy and momentum state will allow this to happen, and this value can be obtained in pair production with some non-zero probability density.

For $f = 1$, equation (4) yields an upper limit on the charge of a black hole of

$$1 \geq (1/E)(m_{ir} + Q^2/4m_{ir}) Q / 2m_{ir} Q_e / m_e \quad (5)$$

Because $Q_e/m_e = 2 \times 10^{21}$ in gravitational units, Q should be small compared to m_{ir} . Thus,

$$1 \geq (Q/2E)(Q_e/m_e) \quad (6)$$

The large magnitude³ of Q_e/m_e yields an upper limit on the electric charge much lower than the limit obtained by Christodoulou and Ruffini¹ except for the case approaching maximal angular momentum, where equation (2) predicts that $Q = 0$. For $L = 0$, Christodoulou and Ruffini obtain $Q \leq 2m_{ir}$, whereas equation (5) yield $Q \leq 2m_{ir}(m_e/Q_e) = 2m_{ir}(5 \times 10^{-22})$, a difference of twenty-two orders of magnitude.

In order to understand the difference between these limits, we proceed by computing the rate of charge loss. We first note that essentially all of the electrons formed by the field should escape because equation (6) is the same equation one obtains in Newtonian gravitation. Thus, the rate of charge loss can be calculated by computing the rate of pair production in the field. Schwinger³ has computed the rate Γ of electron-positron pair production in an electrostatic field as

$$\Gamma = (\alpha^2/\hbar\pi^2)\epsilon^2 \left[\sum_{n=1}^{\infty} n^{-2} \exp(-n\pi m_e^2/q_e\epsilon\hbar) \right] \quad (7)$$

where α is the fine structure constant, ϵ is the electric field, and q_e , the electron charge. For this rate to be significant, $\pi m_e^2/(q_e\epsilon\hbar) \lesssim 1$. If the electric field is estimated as $Q/4M^2$ (in gravitational units), one obtains for the zero angular momentum case)

$$1 \lesssim (\lambda/2\pi M)(Q_e/m_e)(Q/2M) \quad (8)$$

where λ is the Compton wavelength of the electron $\hbar/(m_e c)$.

The case of a 1 solar mass black hole yields $1 \lesssim 1.73 \times 10^5 Q/2M$ for a significant rate. The lowest value of Q for which this rate is significant is thus in this case approximately 10^5 lower than the upper limit computed by Christodoulou and Ruffini. Furthermore, for $Q = 2M$, one can use equation (7) to estimate the rate of charge loss. A conservative estimate is obtained with $\Gamma \sim \alpha^2 c^2 / \hbar \pi^2$. This value of Γ must be multiplied by a volume which we estimate as R^3 and a time dilation factor which is no worse than $\chi/2M$ (this is the gravitational time dilation term at a distance χ from the Schwarzschild radius). This yields a rate for $Q = 2M$ of at least $\alpha^2 c^4 / (\hbar G) = 10^{60}$ electrons s^{-1} provided only that the black hole mass M allows equation (8) to be satisfied with $Q \leq 2M$.

Thus, the difference between the upper limits occurs because equation (6) is a weaker statement than equation (2). The upper bound given by equation (6) can be temporarily violated: charge can be added, however the added charge will be lost if the black hole is left alone. Equation (2), on the other hand, can never be violated. We further note that as the charge becomes larger [for value of Q above the upper limit of equation (6)] more energy is available for the process and f therefore increases above its minimum value. This increase in energy can be distributed arbitrarily between the electron and positron. The electron may have non-zero kinetic energy, and m_{e+} can increase. The phase space restrictions on the ejection process are then less than for smaller charges. This, together with the increase in the pair production rate with increasing charge, causes the increase in the charge ejection rate.

The following physical picture then emerges: starting with an uncharged black hole, one can add charge until the inequality in equation (6) is satisfied. Beyond this point, charge may still be added: however, a net inflow of charge is necessary to balance the loss of charge by the mechanism proposed in this paper. The charge may then be increased until the absolute bound given by equation (2) is reached.

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W. T. ZAUMEN

Lockheed Palo Alto Research Laboratory,
3151 Hanover Street
Palo Alto, California 94304

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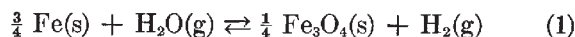
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Meteoritic magnetite as a cosmo-thermometer

Latimer¹ and Urey² first investigated the oxidation states of iron in the dust phase of the solar nebula, demonstrating for the reaction



that the equilibrium constant $K = \text{H}_2/\text{H}_2\text{O}$ is determined by the relative abundance of hydrogen and oxygen in the solar nebula. The equilibrium constant is further related to the standard free energy change, ΔG° , by the well known thermodynamic relation

$$\Delta G^\circ = -RT \ln K \quad (2)$$

Thus, with ΔG° obtained from thermodynamic data and K determined by the solar $\text{H}_2/\text{H}_2\text{O}$ ratio, the equilibrium temperature of ~ 400 K is calculated.

On the basis of the above thermodynamic reasoning, the presence of magnetite in carbonaceous chondrites has frequently been cited as a cosmo-thermometer to infer accretion temperatures of $\lesssim 400$ K (refs 3-5). The equilibrium temperature determined from equations (1) and (2), although providing a 'go-no-go' criterion for the formation of magnetite from the oxidation of metallic iron, yields no information regarding the potential sensitivity of that reaction to local, possibly non-ideal, conditions. In this respect it is instructive to consider the separate equilibria involved in equation (1) which are related by the oxygen fugacity, f_{O_2} . Independent of whether meteoritic magnetite formed from the oxidation of metallic iron as proposed by Urey² or from the oxidation of troilite (J. M. H., unpublished), no loss of generality is expected from this approach as the formation and subsequent stability of magnetite in the early Solar System depends on the prevailing oxygen fugacity.

If we assume in an atmosphere of solar composition that the oxygen fugacity is governed by the gas phase reaction



we can calculate the oxygen fugacity as a function of temperature with the expression

$$-\log_{10}(f_{\text{O}_2}) = -2\Delta G^\circ_f / 2.3RT - 2 \log_{10}(\text{H}_2\text{O}/\text{H}_2) \quad (4)$$

where the ratio $\text{H}_2\text{O}/\text{H}_2$ is determined from cosmic abundance tables⁶ and ΔG°_f is taken from thermodynamic data. We can further calculate the oxygen fugacity equilibria for the reaction



with the expression

$$-\log_{10}(f_{\text{O}_2}) = -\Delta G^\circ_f / 2(2.3)RT \quad (6)$$

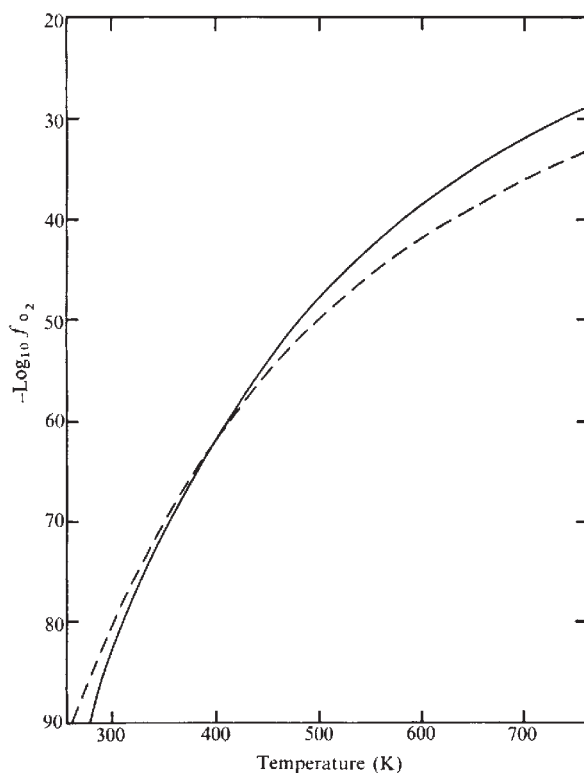


FIG. 1 Fe-Fe₃O₄ oxygen fugacity stability fields and the oxygen fugacity expected as a function of temperature in an atmosphere of solar composition. —, Fe-Fe₃O₄ oxygen fugacity equilibria line. The magnetite stability field lies above this line, metallic iron below. ---, oxygen fugacity in an atmosphere of solar composition.

For temperatures under consideration here, FeO equilibria can safely be ignored.

The results of these calculations are shown in Fig. 1. For temperatures below ~ 400 K the oxygen fugacity as calculated for an atmosphere of solar composition lies within the magnetite stability region. This result is expected as equation (1) actually represents the combination of equations (3) and (5). The significance of these calculations, however, is demonstrated by the near coincidence of the two curves shown in Fig. 1. The Fe-Fe₃O₄ oxygen fugacity equilibria over a temperature range of several hundred degrees lies quite close to the oxygen fugacity expected in an atmosphere of solar composition. The expected oxygen fugacities as calculated are ideal. In the early Solar System only mild local conditions (such as gas phase reactions involving carbon or sulphur) need have occurred to have created oxygen fugacities which were either more oxidizing or more reducing than those calculated. Recognising that the formation and subsequent stability of magnetite is likely to be quite sensitive to local redox conditions over a wide range of temperatures, it seems inappropriate to use the presence of magnetite in meteorites as a cosmo thermometer to infer accretion temperatures of ≤ 400 K.

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J. M. HERNDON
M. W. ROWE

Department of Chemistry
Texas A&M University
College Station, Texas 77843

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Radio emission from R Aquarii

R AQUARI has been known as a variable star since its discovery by Harding in 1811. Although R Aquarii is usually referred to as a long-period variable it has been described as a symbiotic nova by Merrill (ref. 1, page 84) and Payne-Gaposhkin². Photographic evidence discussed below indicates that a nova outburst occurred approximately 600 yr ago. Prompted by the nova-like radio outbursts from Cygnus X-3 we started a search for radio emission from some of the eruptive variable stars. Here we report the detection of variable radio emission from R Aquarii.

The initial detection was made on April 23, 1973 at 10.5 GHz using the 46 m telescope of the Algonquin Radio Observatory. The measured flux density was 95 ± 10 m.f.u. (1 m.f.u. = 10^{-26} W m⁻² Hz⁻¹). On May 19 the star was again observed at 10.5 GHz and the flux density recorded with 22 ± 10 m.f.u. The observations were obtained using the nodding technique³ which uses a main and reference beam separated by 8 arc min in zenith angle. Both observations were made at the same hour angle so that the uncertainty in the measured flux density due to confusion in the two reference beam positions would be the same in each case. At this frequency the principal confusing sources are HII regions in the galactic plane. In view of the large galactic latitude of R

Aquarii ($b^{\text{II}} = -71^\circ$) confusion effects are probably insignificant. The telescope was pointed on the optical position for R Aquarii within 15 arc s on the basis of the telescope pointing tests carried out on both occasions. The half-power beam-width of the telescope is 2.8 arc min at 10.5 GHz.

Further observations were obtained using the Greenbank three-element interferometer at 2,695 and 8,085 MHz during two subsequent periods in June and August 1973. On both occasions a radio source was detected at the two frequencies coincident with the optical position given in the Smithsonian Astrophysical Observatory star catalogue. These results are given in Table 1. The observations for the two periods were combined to obtain synthesis maps of R Aquarii at both frequencies for all 300 m spacings up to 2,400 m. The synthesised beams at the two frequencies are identical except for a factor of three in size. The synthesised beam at 8,085 MHz is shown in Fig. 1 and the 8,085 and 2,695 maps of R Aquarii are given in Figs 2 and 3 respectively. R Aquarii appears to be unresolved at 8,085 MHz which places an upper limit on the source size of ≈ 1 arc s.

TABLE 1 Position and flux densities for R Aquarii obtained with the Greenbank-three-element interferometer

Radio position SAO catalog position	RA (1950)		Dec. (1950)	
	23 h 41 min 14.16 s $\pm$ 0.05 s		-15°33'41.5" $\pm$ 2"	
Date	Interferometer spacings (units of 100 m)		Flux density m.f.u.	Spectral index α
	2,695 MHz		8,085 MHz	
June 20-24	24-21-3		12 $\pm$ 2	-0.3 $\pm$ 0.6
August	24-18-15-12-9-6		8 $\pm$ 1	-0.2 $\pm$ 0.4

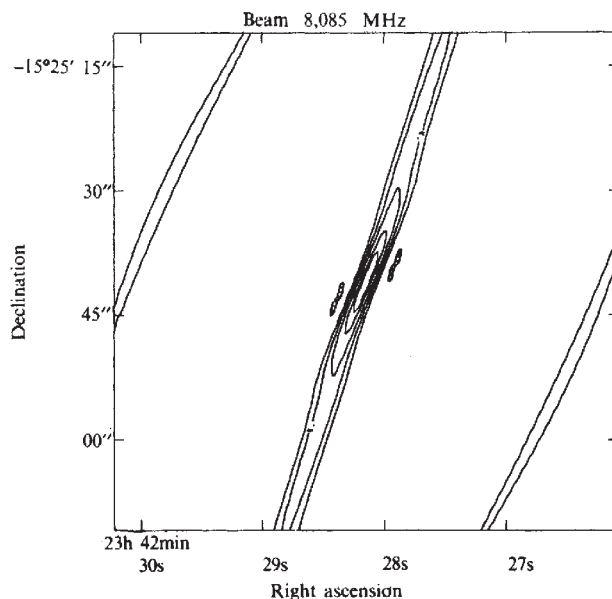


FIG. 1 The synthesised beam at 8,085 MHz obtained with the Green Bank Interferometer. A map of a single unresolved source with a high signal-to-noise ratio should look identical to the beam pattern. Epoch of coordinates given is 1973.6. Field of view 1×1 arc min.

No significant short term variability was detected during the interferometer observations and the flux density measurements at 10,500, 8,085 and 2,695 MHz are consistent with a slow decay in the flux density of R Aquarii throughout

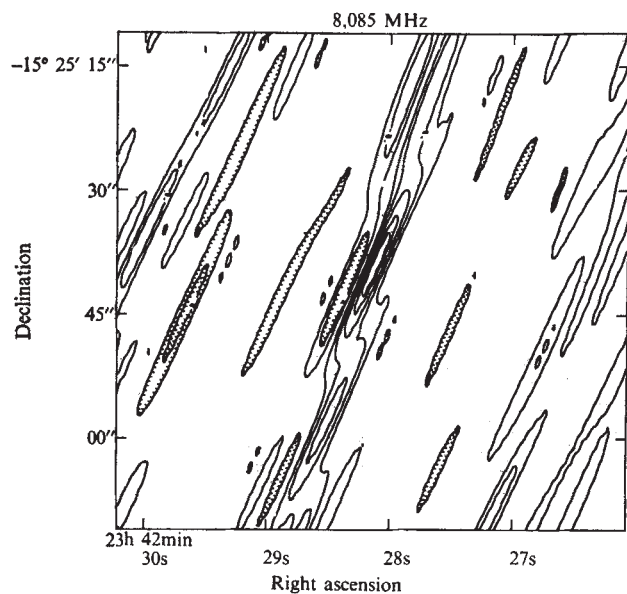


FIG. 2 Map of R Aquarii at 8,085 MHz with single-to-noise ratio of approximately 7:1. Hatched contours represent negative side-lobes. Field of view 1×1 arc min.

the entire period of observations from April to August 1973. The spectral index α as defined by the relationship S (flux density) $\propto \nu^\alpha$ is not significantly different from the value expected ($\alpha \approx -0.1$) for optically thin thermal bremsstrahlung radiation.

According to Merrill (ref. 1, page 84) the optical spectrum of R Aquarii shows evidence for both a red and blue component as well as emission lines characteristic of gaseous nebulae. The red component which is usually the stronger of the two is an M7e long period variable of period 387 d with an apparent magnitude range from 6.7 to 11.6. The spectrum of the blue component exhibits high excitation lines of hydrogen, helium and ionised iron. Merrill also points out that the brightness of the blue component has varied in an irregular manner. Although most of the time at eleventh magnitude or fainter it has been as bright as eighth magnitude. During 1931–33, when the blue component

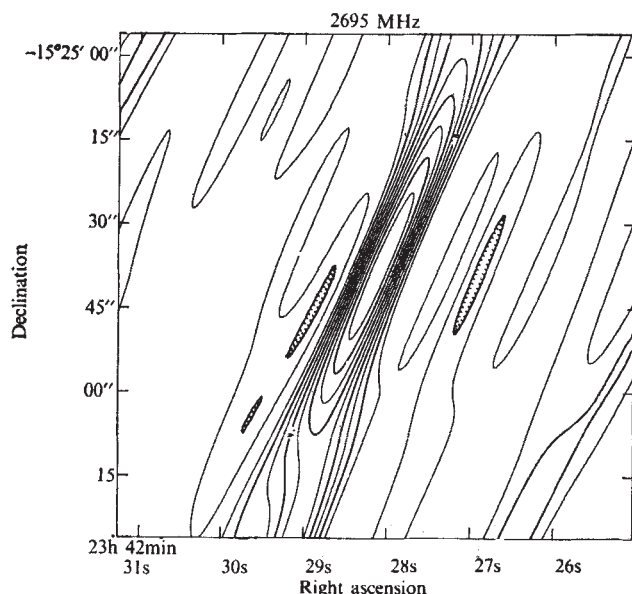


FIG. 3 Map of R Aquarii at 2,695 MHz with signal-to-noise ratio of approximately 9:1. Field of view 1.5×1.5 arc min.

was especially bright, the Me variable exhibited a succession of abnormally low maxima. At this time the spectrum of the blue component was comparable to that of a Bep star.

The evidence suggesting that the blue component is an ex-nova comes from photographs revealing two interesting formations of faint nebular matter surrounding the star (see Fig. 4). The first photograph of R Aquarii was taken in 1921 by Lampland⁴. A summary of the chief characteristics of the nebula⁵ is given below.

The most conspicuous feature is the lens shaped structure formed by two intersecting arcs in which the star is centrally and symmetrically placed. The position angle of the major axis of the arcs is about 90° and the greatest extent of the faint structure is a little more than 2 arc min. The inner nebula which is approximately 15 arc s in angular extent appears to be variable in position or brightness or both.

The outer nebula, although exhibiting no variations in brightness, was detected by Hubble to be expanding. The expansion, which is compatible with a nova-like outburst, was confirmed by Baade⁶. A pair of plates separated by a 16 yr time interval indicated that the nebula was ejected 600 yr ago, on the assumption of uniform expansion. Spectroscopic observations indicated an expansion velocity of 80–100 km s⁻¹, thus placing R Aquarii at a distance of 260 pc (ref. 6). This distance is consistent with the distance derived from the observed maximum visual magnitude of the long period variable.

It is clear from the photograph that R Aquarii is imbedded in a cloud of ionised gas with an angular extent of ~ 15 arc s. The general appearance of the inner nebula suggests that it was formed by the ejection of material from R Aquarii. A possible explanation of the radio emission is that it is optically thin thermal bremsstrahlung radiation from a denser central cloud of ejected matter surrounding the binary system and having an angular extent of < 1 arc s (corresponding to a linear extent of 4×10^{15} cm).

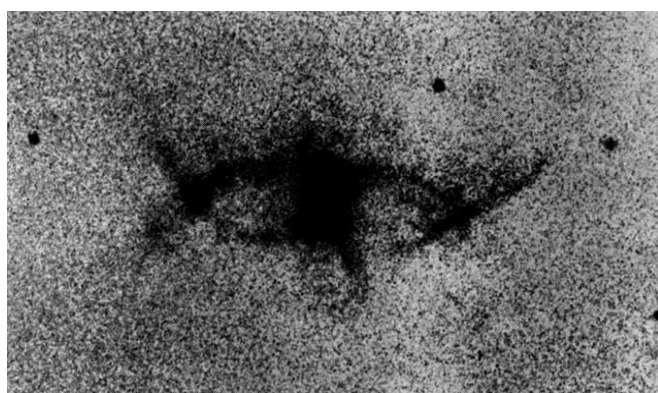


FIG. 4 Photograph of R Aquarii by E. P. Hubble with the Mount Wilson 100 inch reflector, October 12, 1939; exposure 1 h 30 m.

The variable radio emission could then be explained by a sudden ejection of matter by the blue component. The existence of the expanding outer nebula and general appearance of the inner nebula provides ample evidence for the ability of R Aquarii to eject matter.

As one test of this model we can calculate a lower limit on the angular size of the central cloud required to explain the radio emission and compare this with the observational upper limit of 1 arc s. The lower limit is obtained from the following argument. For a thermal source of angular size θ the maximum possible flux density at the frequency ν is

reached when the source is optically thick at this frequency

$$S = (2kT_e \nu^2 / c^2) \theta^2 \quad (1)$$

where T_e = electron temperature and k = Boltzmann's constant. From a knowledge of the optically thick value of S , equation (1) can be used to derive the product of $\theta^2 T_e$. If at the lowest frequency of observation the source is known to be optically thin then the observed flux density will be less than the optically thick value and the application of equation (1) will lead to a lower limit for $\theta^2 T_e$. For our observations of June and August a limit on θ of ≥ 0.5 arc s is obtained assuming $T_e = 10^4$ K. Since this value is less than the observational upper limit of 1 arc s the proposed model is consistent with our observations. In this respect observations at 1,420 MHz with the Westerbork interferometer would be extremely valuable for on the basis of this model we would predict that the central cloud should be optically thick at 1,420 MHz. Finally from the observed value of the optically thin flux density of the central cloud, the condition that $0.5'' < \theta < 1''$ and the known distance to R Aquarii of 260 pc we obtain limits for the r.m.s. electron density of $6 \times 10^4 < N_e < 2 \times 10^6 \text{ cm}^{-3}$ and limits on the mass of ionised gas of $3 \times 10^{-6} < M < 10^{-5} M_\odot$.

In addition to being a very interesting optical and radio source, R Aquarii has a large infrared excess thought to be produced by a dust cloud. At $11.5 \mu\text{m}$ the infrared flux density of R Aquarii⁸ is $1.85 \times 10^{-23} \text{ W m}^{-2} \text{ Hz}^{-1}$. The ratio of the dust cloud emission peak to the cool star continuum is typical of other Mira variables such as o Ceti and X Cyg⁸ so that presumably the dust cloud is associated with the red component of R Aquarii.

The dates of the radio observations fall close to the expected minimum in the light curve of the red component of R Aquarii. From the optical data of the American Association of Variable Star Observers, kindly supplied to us by Margaret Mayall, there is no evidence of unusual optical behaviour immediately prior to or after the radio observations. Unfortunately there is an absence of optical data during April and May. Further observations are planned to examine the relationship between the radio and optical behaviour of R Aquarii.

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P. C. GREGORY*
E. R. SEAQUIST

David Dunlap Observatory,
University of Toronto,
Richmond Hill, Ontario

Received December 28, 1973.

* Present address: Physics Department and Institute for Astronomy, The University of British Columbia, Vancouver, BC, V6T 1W5.

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Fluctuations in oil flow before and after earthquakes

INTEREST has been growing recently in geophysical phenomena which occur near the time of earthquake events. We have been observing the flow of oil from wells in the Gulf of Suez and have noticed that remarkable fluctuations in flow occurred near the time of certain nearby earthquake events. The Gulf of Suez is considered to be an opening tensile region with Sinai moving away from the African continent¹. The earthquakes occurred close to the bifurcation point of the Gulf of Suez and Eilat and the wells are about 100 km north from this point in the Gulf of Suez (Fig. 1, insert). The oil was driven up by natural pore pressure and was not pumped. Measurements of oil flow were made once a month. Figure 1 shows that from about January 1968 to June 1969, and from about April 1971 to July 1972, large fluctuations occurred in oil outflow. Also, within these periods and in particular towards the end, several moderate earthquakes occurred. In the first period there was an event on March 31, 1969 (magnitude

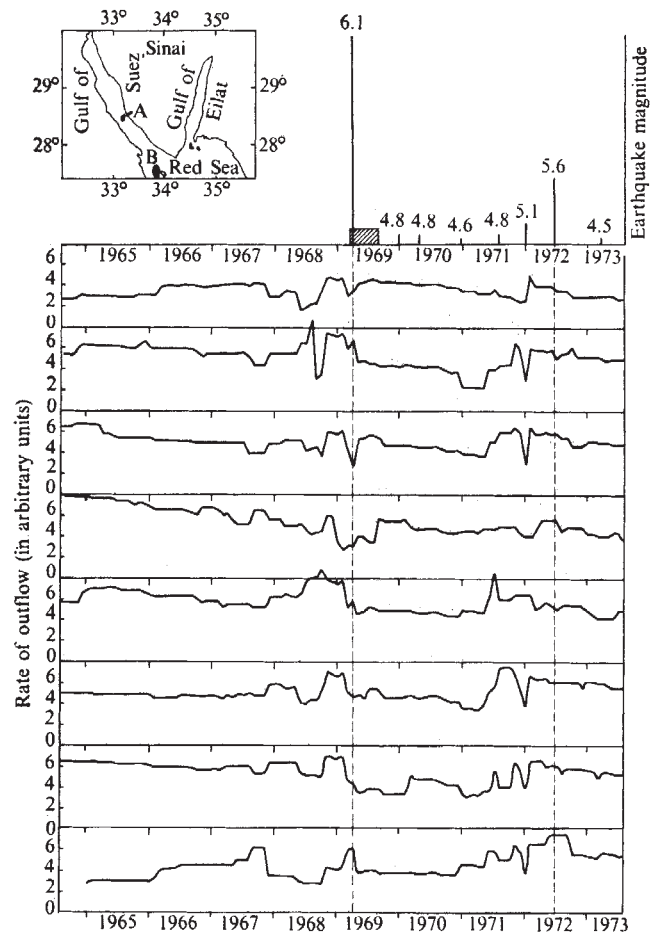


FIG. 1 Measurements of oil flow from wells in the Gulf of Suez, July 1964 to July 1973. Earthquakes are indicated above together with their magnitude. The shaded region under the 6.1 magnitude earthquake indicates foreshocks. Note the similarity of time function starting before the 1969 earthquake (magnitude 6.1) and the 1972 earthquakes (magnitudes 5.1 and 5.6). Inset, Map of Sinai region; A, Location of oil wells; B, Location of earthquakes.

6.1) plus a few foreshocks and many aftershocks reaching a maximum magnitude of 5.0. In the second period there were events on July 8, 1971 (magnitude 4.8), January 12, 1972 (5.1) and June 28, 1972 (5.6). Except for these periods the oil outflow was on the whole more stable and the earthquakes that did occur were of smaller magnitude, the largest ones being of magnitude 4.8 on December 30, 1969 and April 28, 1970. (No earthquakes occurred from January 1964 to February 1969.) The choke openings at the tops of the pipes were varied from time to time but after examination it seems to us that these variations did not cause the observed fluctuations.

If the simultaneous occurrence of flow fluctuations and moderate earthquakes is not coincidental, what is the possible connection between them? Pre-seismic deformations seem to occur over fairly large distances² and are coupled to changes in pore pressure³⁻⁵. The data suggest to us that a pre-seismic deformation may have occurred around the site of the impending earthquake and that its influence extended as far as the oil wells. The deformation caused changes in pore pressure which gave rise to the large fluctuations in oil flow.

Clearly the evidence presented here is not conclusive. An attempt at detailed explanation of the phenomena has to take into consideration, among other things: (1) that there are some fluctuations in oil flow outside the two periods and (2) that, within the two periods, not all the fluctuations can be correlated with one another. With the present interest in pre-earthquake phenomena, however, we felt that these data should be made known. It would be interesting to see if such phenomena occur in other regions, since they may help in the prediction of earthquakes.

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E. ARIEH
A. M. MERZER

*Seismological Laboratory,
Geological Survey of Israel, Jerusalem
Department of Environmental Sciences,
University of Tel Aviv*

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Alternative interpretation of sulphur isotope ratios in the McArthur lead-zinc-silver deposit

SMITH and Croxford¹ have reported important new measurements of variations among the isotopes of sulphur in the enigmatic stratiform sulphide deposit at McArthur River, Northern Territory, Australia. The data place severe constraints on processes capable of forming the deposit, and Smith and Croxford interpret them to indicate a syn-sedimentary origin with sulphur supplied from two separate sources—one for pyrite and one for galena and sphalerite. We suggest that Smith and Croxford have misinterpreted several important aspects of their data and that they have thereby been led to erroneous conclusions. The isotopic data

do not really support their preferred models of mineralisation. Rather, they support the kind of single-sulphur source model demonstrated for many other stratiform base-metal sulphide deposits, in which dissolution of pre-existing pyrite by a circulating, metal-rich but sulphur-poor brine is followed by deposition of base-metal sulphides. Brines of the kind capable of forming such deposits have been reported from the Salton Sea geothermal field², from the Cheleken region in Russia³, in oil fields⁴ and in fluid inclusions⁵. The most complete elucidation of such a model is that by Brown⁶ for the White Pine deposit in Michigan but the applicability of the model to many deposits has been pointed out by White⁷ and others.

Smith and Croxford observed isotopic fractionations between sphalerite and galena ranging from 2.8‰ to 5.7‰. They interpreted the fractionations to mean that isotopic equilibrium was approached, or attained, between an average temperature of 170° C and 220° C. The interpretation is misleading. If, indeed, isotopic equilibrium were attained, the fractionations indicate a temperature range of at least 100° C to 260° C⁸. Equilibrium fractionations in excess of 4‰ have, however, never been achieved under controlled experimental conditions and it has been pointed out by Czamanske and Rye⁹ that large and variable fractionations are probably indicative of low temperatures (200° C or less) of formation and isotopic disequilibrium.

Smith and Croxford also reported that $\delta^{34}\text{S}_{\text{pyrite}}$ increases upward through the stratigraphic section whereas $\delta^{34}\text{S}_{\text{galena}}$ and $\delta^{34}\text{S}_{\text{sphalerite}}$ seem to be independent of stratigraphic position. This led them to propose their dual sulphur reservoir model. With respect to pyrite, their observation is typical of, and compelling evidence for, pyrite formed diagenetically in anoxic sediments with sulphur supplied by bacterial reduction of sulphate within a closed system¹⁰. The failure of $\delta^{34}\text{S}_{\text{galena}}$ and $\delta^{34}\text{S}_{\text{sphalerite}}$ to mimic the $\delta^{34}\text{S}_{\text{pyrite}}$ trend, however, does not necessarily indicate a separate source for sulphur; it may simply reflect a different mode of mineral deposition.

The average value of $\delta^{34}\text{S}_{\text{pyrite}}$ in the deposit is +6.1‰. Assuming isotopic equilibrium between solution and precipitate, the $\delta^{34}\text{S}$ range for H_2S in a brine capable of depositing the McArthur sphalerite and galena is 4‰ to 8‰^{5,11}. Although recognising that the data do not indicate isotopic equilibrium, we suggest that the actual isotopic composition of the brine was probably within or close to the suggested range. If, therefore, dissolution of pyrite served to supply sulphur to a circulating brine, the isotopic composition in the resulting fluid would be just about that expected to yield the $\delta^{34}\text{S}$ values observed for galena and sphalerite.

If our interpretation of Smith and Croxford's measurements is correct, we would expect to see a systematic relationship between $\delta^{34}\text{S}$ in each of the sulphides. In Fig. 1 we have plotted $\delta^{34}\text{S}$ for each sulphide against its stratigraphic position (distance above footwall). The distribution of $\delta^{34}\text{S}$ of each sulphide about an average line fitted by least squares is striking, and particularly noticeable in the upper and lower parts of the stratigraphic section. We suggest that this striking similarity of local variations of $\delta^{34}\text{S}$ means that the sulphides must be genetically related. We therefore propose the following model as the one most nearly accounting for the various observations reported on the McArthur deposit.

The host shales within which the deposits are now found were normal anoxic sediments in which were formed the thin laminae of pyrite, the pyrite framboids and the pyritized sedimentary grains reported by Croxford and Jephcott¹². The pyrite was formed during diagenesis with sulphur supplied by bacterial reduction of sulphate in a closed reservoir. We suggest that the original upward enrichment of $\delta^{34}\text{S}$ was even more pronounced than that now seen in the McArthur deposit—possibly as pronounced as

the line BB in Fig. 2. Subsequent to the formation of the pyrite, a base-metal-rich but sulphur-poor brine system was

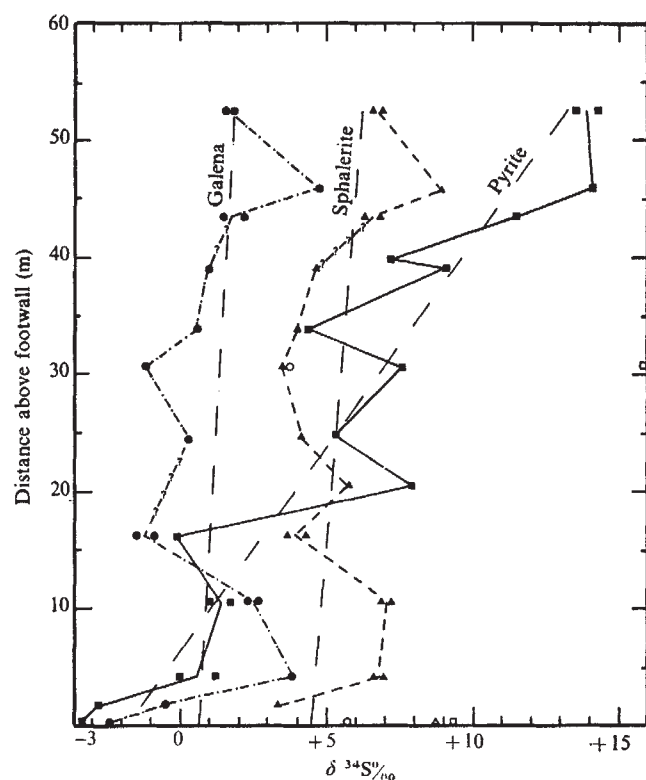


Fig. 1 Variations in the sulphur isotopic composition of galena, sphalerite and pyrite in the McArthur orebody. ●, Galena; ▲, sphalerite; ■, pyrite; — — —, least squares fit line. Solid symbols represent shale mineralisation and open symbols represent slump breccia mineralisation.

set in motion. A local heat source and nearby evaporite deposit, both helpful but not essential prerequisites for the origin and movement of such a brine, were present at McArthur River^{12,13}. The brine was initially undersaturated with respect to pyrite and therefore dissolved some of the pyrite from the shale to produce an isotopically homogeneous solution with a $\delta^{34}\text{S}$ close to 6‰. As dissolution of pyrite proceeded the brine became saturated with respect to galena and sphalerite, and both minerals commenced precipitating within the shales. Concurrently, other shale-brine reactions (such as those involving silicates and carbonates) were taking place, altering the chemistry of the brine and its solution properties. Eventually a second generation of pyrite was precipitated around the remaining grains of diagenetic pyrite to produce the distinctive and ubiquitous brown-yellow overgrowths reported by Croxford and Jephcott^{12,14}. The $\delta^{34}\text{S}_{\text{pyrite}}$ values reported by Smith and Croxford therefore must be an average of $\delta^{34}\text{S}_{\text{diagenetic pyrite}} + \delta^{34}\text{S}_{\text{overgrowth pyrite}}$. The marked stratigraphic trend of $\delta^{34}\text{S}_{\text{pyrite}}$ observed by Smith and Croxford, therefore, reflects the effect the sulphur in diagenetic pyrite had on their measurements, and the similarity in distribution between $\delta^{34}\text{S}$ for pyrite, galena and sphalerite reflects the influence of the overgrowth pyrite drawing sulphur from the same reservoir as the galena and sphalerite.

An idealised behaviour of sulphur isotopes predicted by our model is presented in Fig. 2. Line AA is the observed stratigraphic trend for $\delta^{34}\text{S}_{\text{pyrite}}$. Line BB is a postulated $\delta^{34}\text{S}$ trend for the original diagenetic pyrite distribution. CC is the $\delta^{34}\text{S}$ of an overgrowth pyrite precipitated from the

same solution as galena and sphalerite at an average temperature of 170°C. No real significance should be attached to this temperature, because it is not possible to assign an accurate value to the temperature at which the reactions actually occurred. Qualitatively, however, any low temperature will give a similar result, assuming that the $\delta^{34}\text{S}$ of pyrite reflects the $\delta^{34}\text{S}$ of H_2S in solution. By adding various amounts of overgrowth pyrite to diagenetic pyrite, actual $\delta^{34}\text{S}_{\text{pyrite}}$ stratigraphic curves can be generated. To illustrate this we have shown two such model curves on Fig. 2, one assuming mixing of 20 weight % of overgrowth pyrite and 80 weight % of diagenetic pyrite

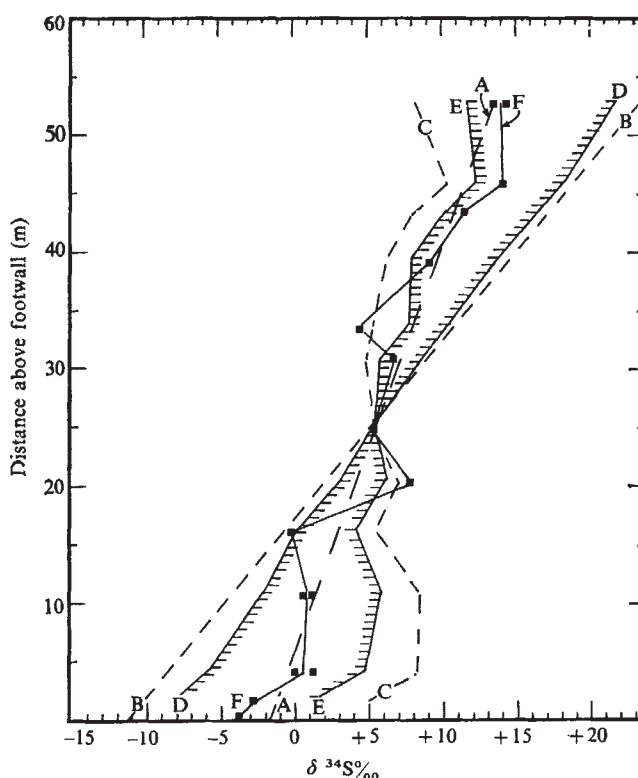


Fig. 2 Variations in the model sulphur isotopic composition of pyrite. AA, Least squares fit line to observed pyrite $\delta^{34}\text{S}$ distribution; BB, postulated diagenetic pyrite $\delta^{34}\text{S}$ distribution; CC, postulated overgrowth pyrite $\delta^{34}\text{S}$ distribution; DD, predicted model pyrite $\delta^{34}\text{S}$ distribution assuming mixing of 20 weight % overgrowth pyrite with 80 wt. per cent diagenetic pyrite; EE, predicted model pyrite $\delta^{34}\text{S}$ distribution assuming mixing of 80 weight % overgrowth pyrite with 20 weight % diagenetic pyrite; F — — — F, observed pyrite $\delta^{34}\text{S}$ distribution.

(curve DD) and the other assuming mixing of 80 weight % of overgrowth pyrite and 20 weight % of diagenetic pyrite (curve EE). All but two points on the observed $\delta^{34}\text{S}$ stratigraphic curve FF lie within the shaded area defined by these model curves, thereby suggesting that our assumed original $\delta^{34}\text{S}$ distributions are reasonable and that the actual amount of diagenetic and overgrowth pyrite mixing at McArthur River is between the limits defined by the curves DD and EE. The two points on FF that are not accounted for by our idealised model can be easily accommodated within the shaded field by allowing for small deviations in the original $\delta^{34}\text{S}$ values about curve CC.

Apparently as an afterthought, Smith and Croxford¹ proposed a qualitatively interesting model that turns out to be mathematically identical to our model. In this model they suggest that iron had an exhalative origin and was initially deposited as a monosulphide together with galena and

sphalerite. Subsequently, the monosulphide was converted to pyrite by reaction with sulphur provided by bacterial reduction of soluble sulphate. This model can be quantitatively described by Fig. 2. Line CC would then represent the $\delta^{34}\text{S}$ distribution of the early monosulphide and BB would represent the $\delta^{34}\text{S}$ distribution of the bacterially produced sulphur. Although mathematically equivalent to our model, this syn-sedimentary model is not texturally equivalent because it cannot satisfactorily explain the pyrite overgrowths present in the McArthur ores^{12,14}.

We believe that our model offers a possible explanation for other geological features found at McArthur. For example, the predominance of iron-bearing dolomites in the unmineralised sediments stratigraphically above and below the deposit¹ would, in our model, represent remobilised iron that was leached from the shales during the mineralising process. Insufficient data, however, prevent us from commenting on the application of our model to the $\delta^{34}\text{S}$ ratios from the slump breccia mineralisation.

If the overgrowth pyrite could be separated from diagenetic pyrite it would be possible to test our model. The model predicts that near the footwall sulphur from the overgrowth pyrite should be isotopically heavier than the sulphur from the diagenetic pyrite, whereas near the hangingwall the opposite should be the case.

We suggest, in conclusion, that the sulphur isotopic ratios from the McArthur lead-zinc-silver deposit are better explained by a single-sulphur source, two-stage mineralisation model than by any of the previously suggested dual-sulphur, syn-sedimentary models.

NEIL WILLIAMS
DANNY M. RYE

Department of Geology and Geophysics,
Yale University,
New Haven, Connecticut 06520

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which have been observed to date and indicate something about the frequency of the various structures.

The basic structural unit of tin sulphide polytypes consists of two layers of hexagonal close-packed sulphide ions with smaller tin ions nested between them. The arrangement is isotypic with other layered MX_2 compounds, for example, $\text{Mg}(\text{OH})_2$, CdI_2 , PbI_2 , and can be represented in a similar manner by A_γB (or B_γA , $\text{A}\beta\text{C}$, $\text{C}\beta\text{A}$, $\text{B}\alpha\text{C}$ or $\text{C}\alpha\text{B}$) in which sulphide ions are represented by Roman letters and tin ions by Greek letters in the common notation for the closest packing of spheres. Various stacking arrangements of this unit 'sandwich' layer can produce different polytypes. For example, *type 2H* is $[(\text{A}_\gamma\text{B})]_n$ and *type 4H* is $[(\text{A}_\gamma\text{B})(\text{C}\alpha\text{B})]_n$. In the polytype name the integer refers to the number of sulphur layers in the unit cell and *H* and *R* indicate hexagonal or rhombohedral symmetry.

Until now *type 2H* has been considered to be the 'normal' SnS_2 structure, with $[(\text{A}_\gamma\text{B})]_n$, $P\bar{3}m1$, $a = 3.6486 \text{ \AA}$, $c = 5.8992 \text{ \AA}$ (ref. 2). This structure was determined first by Oftedal^{3,4}, for synthetic material, and later was recognised as the usual structure of natural tin sulphide (the mineral berndtite) by Moh and others⁵⁻⁷. About a quarter of our crystals are *2H*, second only to *18R* in frequency.

Type 4H has been recognised relatively recently. Structural data for this include $[(\text{A}_\gamma\text{B})(\text{C}\alpha\text{B})]_n$, $P6_3mc$, $a = 3.6486 \text{ \AA}$, $c = 11.7984 \text{ \AA}$. On X-ray powder photographs Guenter and Oswald¹ found evidence for the *4H* structure in synthetic materials, and Clark^{8,9} detected it in natural berndtite. We have found six crystals of pure *4H* and five other crystals in which *4H* is syntactically coalesced with either *18R* or disordered structures.

Type 18R is the most common structure encountered in our crystals. Out of ninety crystals examined, 38.9% are pure *18R*, while additional *18R* structures are coalesced with other polytypes such as *2H* and *4H*. Disordered structures also usually approach *18R* according to our X-ray films. This polytype was identified earlier using the oscillation X-ray method¹⁰ and its structure is being determined by Inoue, Ishizawa, and Fujiki (in preparation).

Two specimens each of *8H* and *10H* have been encountered in our investigation. Their structures will be reported subsequently. The possibility of a *10H* polytype had been suggested by Karakhanova *et al.*¹¹ from studies of X-ray diffraction powder patterns but their data were not convincing.

Other crystals, for which there is some uncertainty about the number of layers in the unit structure, are near *14H*, *24H* (or *72R*) and *30H* (or *90R*).

Fourteen of the ninety crystals exhibit more or less random stacking of the layers (disordered structures) normal to the *c* axis. Most of these, however, apparently contain large 'blocks' of the *18R* structure, as indicated by nodes of higher intensity at the *18R* X-ray reflection positions on the continuous streaks on Weissenberg films.

The crystals used in this study were grown by a chemical transport reaction described earlier¹². The materials used include metallic tin, powdered sulphur, and flake-like iodine. The iodine is used as a transport reagent. The reactions were carried out in vacuum-sealed vycor tubes 13 cm long and 1.1 cm inside diameter. Crystals were grown during transport from the 700° C zone to the 600° C zone. Reaction time ranged from 68 to 142 h. Iodine concentrations varied from 0 to 4 mg cm⁻³. The crystals are golden-yellow to reddish-brown plates, varying from a few millimeters to 15 mm across. They are tabular parallel to {0001} and usually have an anhedronal outline, although hexagonal symmetry can be seen on some of the smaller specimens.

In addition to determining the structures of some of the polytypes mentioned here, we plan to study the relationships between the conditions of growth and the polytypes formed and to consider the possible influence of screw dislocations upon the generation of polytypes. Growth

Structural polytypism of SnS_2

ALTHOUGH recent work has indicated that more than one structural type of tin sulphide (SnS_2) exists¹, the magnitude of polytypism in this substance has not been studied until now. We have recently grown crystals of tin sulphide and have studied Weissenberg X-ray diffraction photographs of ninety specimens. Here we report the several polytypes

spirals, believed to be related to structural polytypism, have been observed on crystals of the compound¹².

RICHARD S. MITCHELL

Lewis Brooks Museum,
University of Virginia,
Charlottesville, Virginia 22903

YOSHINORI FUJIKI
YOSHIO ISHIZAWA

National Institute for Researches in Inorganic Materials,
Kurakake, Sakura-Mura,
Niihari-Gun, Ibaraki 300-31,
Japan

Received November 8, 1973.

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FIG. 1 Locality map, showing the distribution of the Transvaal Dolomite.

record between the Gunflint Formation and those of the Fig Tree Group.

Environmental studies in progress in the Transvaal Dolomite have included a re-assessment of Young's Hol River section, now referred to as the Boetsap River section¹⁸. In this section stromatolitic assemblages dominate an outcrop thickness of no more than 135 m near the base of the Dolomite. A conceptual model has been developed that represents a modification of a limestone shelf model¹⁹, and also an extension of the concept that domes increase in size outward in the intertidal^{20,21} beyond this zone into deeper water. It is inferred that accumulation took place through a telescoped range of environments shelving relatively steeply through the intertidal to a high energy agitated zone and into the subtidal. In this model the more striking columnar stromatolites are intertidal, but seaward of the agitated zone more delicate columns may occur within large domes. We note that the assemblage of as yet unstudied, that is unreconstructed, columnar stromatolites from this locality seems

Filamentous algae from the 2,300 m.y. old Transvaal Dolomite

THE suggestion that stromatolites are analogous with modern algal mats was first made in 1933 (ref. 1). The earliest acceptance of this analogy was for structures in the Transvaal Dolomite². Young described these structures in a series of papers between 1928 and 1943, most of his work being from a spectacular section in the Hol River. This lies 1½ km north of Boetsap, some 50 km north-west of Warrenton in the northern Cape Province (Fig. 1).

Many examples of microfossils are now known from the Precambrian³. On present evidence eukaryotic forms extend back in time through the Bitter Springs Formation⁴ to the Beck Springs Dolomite of age 1,200 to 1,400 m.y. (ref. 5). Prior to this there are examples of rocks containing structures analogous to filamentous blue-green algae as old as the Gunflint Formation⁶, for which an age of ~2,000 m.y. is suggested⁷. Before this there is a gap in evidence of microorganisms back to the Swaziland rocks of the Onverwacht and Fig Tree Groups, which are more than 3,200 m.y. old^{8,9}. The Onverwacht contains paired spheroids and filaments of uncertain biologic affinity^{10,11}, the Fig Tree rod-shaped bacteria^{12,13}.

The Transvaal Dolomite is older than $1,954 \pm 30$ m.y., a date for the granites of the Bushveld Complex¹⁴ post-dating the basic phase, the latter being intruded into strata above the Transvaal Dolomite, and younger than $2,300 \pm 100$ m.y., an age obtained for a single specimen from the underlying Ventersdorp Lavas¹⁵. Recently Crampton (personal communication) dated the Ongeluk lavas above the Dolomites at $2,224 \pm 21$ m.y. The unit containing these lavas is, however, separated from the Dolomites by an unconformity of regional extent^{16,17} suggesting that this is a minimum age with a figure approaching 2,300 m.y. seeming more likely. The present study fills in part of the gap in the microfossil

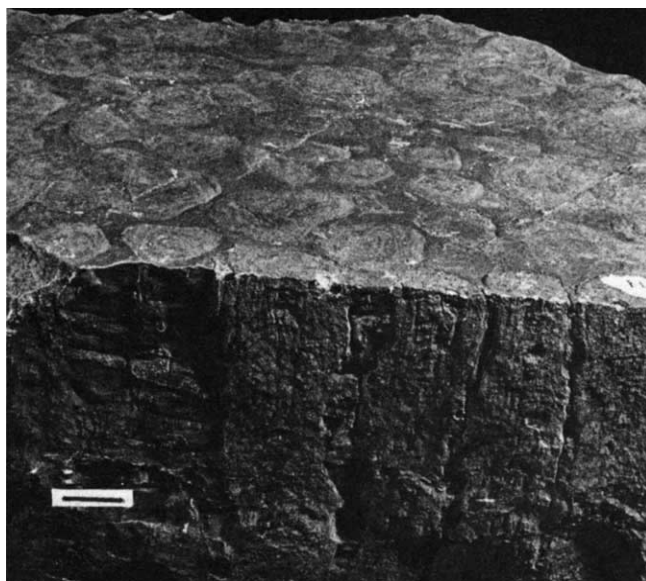


FIG. 2 Intertidal columnar stromatolites. (Scale represents 2 cm).

more diverse than any other pre-Riphean forms described to date. But the analogy of ancient stromatolites and algal mats suggests the presence of microorganisms at the time of formation of any stromatolitic unit, and the purpose of this communication is to document the existence of algal filaments found in association with both subtidal and intertidal columnar forms.

Scanning electron microscopy (by M. MacG.) has revealed the presence of filamentous forms from the Boetsap section. The first of these is recorded from cylindrical columnar stromatolites from the intertidal zone (Fig. 2). The columns are of limestone, the inter-column fill of dolomite. The filaments are narrow, elongate and non-septate. They have a breadth of 1 to 2.5 μm , and a length that may exceed 120 μm (Fig. 3a). A looped filament is illustrated in Fig. 3b.

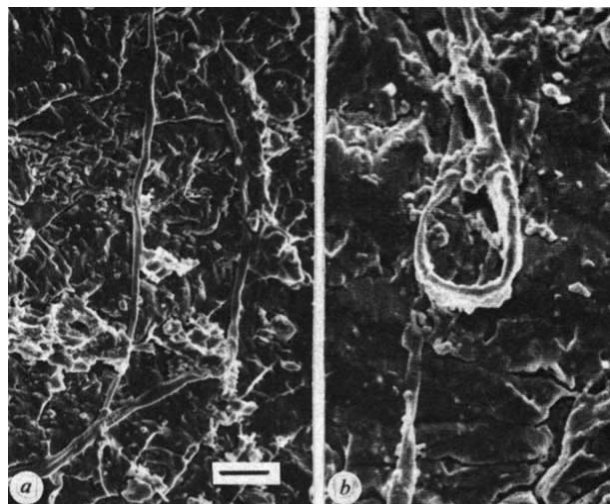


FIG. 3 a, Sheathed algal filament. (Scale represents 10 μm). b, Looped sheathed algal filament. (scale represents 10 μm).

Two other forms are from small-scale columns (Fig. 4) developed in large elongate subtidal domical stromatolites. The columns are composed of interlayered dolomite and limestone, the inter-column fill of limestone. Figure 5a illustrates a slightly curved filament some 70 μm long, tapering at one end from a breadth of 9 μm . Crosswalls are distinct, and the multicellular filament has cells 9 to 14 μm long. The other form is a tapering multicellular filament with an enlarged basal cell (Fig. 5b). The basal cell may represent a heterocyst. There are constrictions at the septae, and the filament is curved. It is 160 μm long, and the average breadth

is 8 μm . The length-to-breadth ratio of the cells is constant, except for the pointed terminal cell.

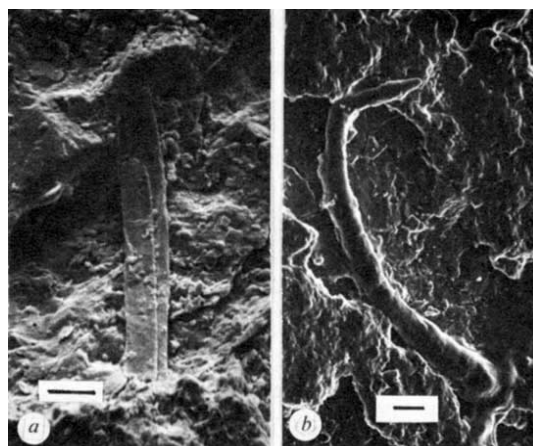


FIG. 5 a, Tapering multicellular filament. (Scale represents 10 μm). b, Tapering multicellular filament with heterocyst. (Scale represents 10 μm).

The filaments shown in Fig. 3a and b, although not displaying any cellular structure, share some characteristics with modern sheathed *Oscillatoriaceae*²². The similarity to modern forms of photosynthetic blue-green algae is more distinct for the filaments illustrated in Fig. 5a and b: the former with a filamentous multicellular form, and no heterocysts or akinetes, to the *Oscillatoriaceae*; and the multicellular, curved tapering form of the latter, with an enlarged basal cell, to the *Rivulariaceae*. In this announcement no attempt is made to further characterise these forms, although Fig. 5b appears similar to the genus *Rivularia*.

The *Oscillatoriaceae* and *Rivulariaceae* are represented in the somewhat younger Gunflint cherts²³, but these Transvaal forms are the oldest blue-green algae from these two families yet recorded. The Transvaal Dolomite is among the oldest extensive shelf carbonates known, that is as distinct from more localised carbonates associated with volcanicity. If there is a relationship between the accumulation of free oxygen on a large scale and the accelerated precipitation of carbonate sediments²⁴, then it can be speculated that photosynthetic organisms may not have developed in any great abundance much prior to about 2,300 m.y.

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I. M. MACGREGOR
J. F. TRUSWELL
K. A. ERIKSSON

Bernard Price Institute for
Palaeontological Research,
and Department of Geology,
Department of Geology,
University of the Witwatersrand, Johannesburg

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FIG. 4 Subtidal columnar stromatolites. (Scale represents 2 cm).

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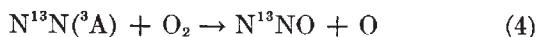
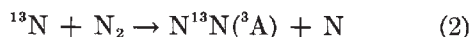
New route for the quenching of $N_2(A^3\Sigma_u^+)$ in the aurora?

THE volume production rate of $N_2(A^3\Sigma_u^+)$ in the aurora, as estimated from the observed emission rates in the N_2 first positive and second positive systems¹, is an order of magnitude greater than the observed emission rate² in the Vergard-Kaplan system³ ($A^3\Sigma_u^+ - X^1\Sigma_g^+$). This implies a high rate of quenching by other species present in the auroral region. The most prominent species at the appropriate altitudes (>100 km) are N_2 , O_2 , N , O and NO (ref. 4). Detailed analysis of the quenching rate has led to the prediction that collision with O atoms makes a major contribution to the quenching process. However such quenching in the aurora would have to proceed¹ with an efficiency one or two orders of magnitude greater than observed in the laboratory^{5,6} in order to account for the observed V-K intensities.

It has been proposed⁷ that one reason for this apparent discrepancy may be errors in the laboratory determination of O atom concentrations. Even so it is clear that our present understanding of the quenching mechanism is incomplete^{1,2}.

At the University of Kent we have been studying some reactions of gas phase nitrogen atoms, using the nuclear process $^{14}N(n, 2n)^{13}N$ (with ~ 14 MeV neutrons produced by a SAMES neutron generator). We have observed that in mixtures of N_2 and O_2 , ^{13}N forms only two products, $N^{13}N$ and $N^{13}NO$, which are detected by radiogaschromatography. Fractional yields of $N^{13}NO$ obtained at various oxygen mole fractions are shown in Table 1. It is clear from these results that $N^{13}NO$ is formed readily in the presence of minimal quantities of oxygen.

We believe that these results are consistent with the following elementary reactions:



The assignment of the $A^3\Sigma_u^+$ state to the electronically excited species proposed for these reactions is consistent with, first, the very slow quenching rate of $A^3\Sigma_u^+$ in N_2 ($k \sim 10^{-20}$

TABLE 1 Fractional yield of $N^{13}NO$ at various O_2 mole fractions in N_2/O_2 mixture.*

O_2 mole fraction	Fractional yield of $N^{13}NO$
0.51	0.48 ± 0.05
0.25	0.48 ± 0.05
0.12	0.42 ± 0.05
$\sim 10^{-6} \dagger$	$0.19 \pm 0.05 \ddagger$

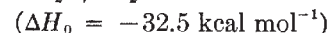
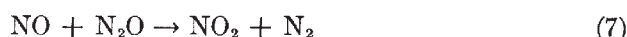
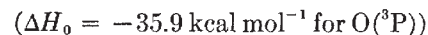
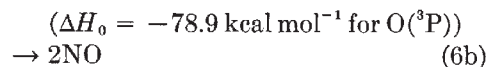
* All results refer to N_2/O_2 mixtures at a total pressure of 5 atm in a 20 ml reaction vessel.

$\dagger$ This figure is intended as an order of magnitude indication only. The gas was commercial "oxygen free nitrogen" (BOC Ltd).

$\ddagger$ This figure represents an average value obtained from several different samples of N_2 , obtained from different gas cylinders and irradiated in different reaction vessels.

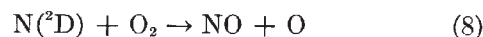
$\text{cm}^3 \text{s}^{-1})^8$ and, second the high radiative lifetime of $A^3\Sigma_u^+$, ($\tau \sim 2 \text{ s}$)³.

If this assignment is correct, then reaction (4) becomes an exoergic process, the amount of energy released depending on the product states. If these are $N_2O(X^1\Sigma^+)$ and $O(^3P)$, then the energy released is about 70 kcal mol⁻¹. N_2O formed in the aurora by the mechanism proposed above would be expected to disappear fairly rapidly. Indeed mass spectrometer measurements have indicated only small amounts of mass 44 species in the thermosphere⁹ (and resolution into CO_2 and N_2O has not yet been confirmed) and only small quantities of N_2O are found in the lower stratosphere¹⁰. Apart from photochemical destruction, possible chemical reactions which would result in the removal of N_2O are reactions (5) to (7).



These reactions have been studied under laboratory conditions¹¹⁻¹³.

It seems likely that $N(^2D)$ is the precursor of much of the NO formed in the mesosphere and thermosphere¹⁰. Recent measurements of the rate constants for (5) and (8), at $\sim 300^\circ K$,



indicate that the two processes are of comparable efficiency ($k \sim 4 \times 10^{-12} \text{ cm}^3 \text{s}^{-1}$)¹⁴, which is surprising in view of the large number of highly exothermic adiabatic channels open to reaction (5). Experimental determination^{12,13} of the activation energies for reactions (6), involving ground state $O(^3P)$ atoms, (14.5 and $> 15.5 \text{ kcal mol}^{-1}$ respectively) and reaction (7) ($\sim 50 \text{ kcal mol}^{-1}$) suggest that these reactions may be less favourable on energetic grounds, although recent rocket measurements⁴ of O and NO indicate considerably greater densities of these species than had previously been contemplated. The prominence of the oxygen ($^1S - ^1D$) transition in the aurora¹⁵ suggests that one should also consider reactions (6) with $O(^1D)$ atoms, in which instance the reaction efficiency is greatly increased, ($k \sim 2 \times 10^{-10} \text{ cm}^3 \text{s}^{-1}$)¹⁴.

These considerations suggest that N_2O may be one of the species produced in the aurora as a result of the chemical reaction of $N_2(A^3\Sigma_u^+)$ with oxygen. A number of chemical processes, as well as photodissociation, are expected to result

in the rapid destruction of any N_2O formed. Nevertheless a measurement of the N_2O density in the aurora would be most welcome.

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D. J. MALCOLME-LAWES

University Chemical Laboratory,
Canterbury, Kent

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BIOLOGICAL SCIENCES

Model for the Secondary Structure of the Denatured Conformer of Yeast tRNA_{3^{Leu}}

YEAST tRNA_{3^{Leu}} is one of the more thoroughly investigated tRNA species because it can exist in two conformations which can be interconverted¹⁻⁵. The native (N) conformer has full biochemical activity, whereas the denatured (D) conformer is inactive with respect to aminoacylation. Although other tRNAs can be reversibly denatured⁶⁻⁸, the D conformer of yeast tRNA_{3^{Leu}} is quite stable¹⁻⁵. Because of these properties, yeast tRNA_{3^{Leu}} has been studied in the hope that it might provide insight into the synthetase recognition problem. This aim has been thwarted, however, by lack of information on the secondary structure of the D conformer. Even less is known about the tertiary structure of this molecule in solution.

Fresco noted that both the DHU and T ψ C loops have regions complementary with the bases in the anticodon loop, and suggested that in the D conformer the anticodon loop is hydrogen-bonded, possibly to a complementary sequence in the T ψ C loop⁶. This possibility, and disruption of the DHU stem, are the only two structural features which were specified for the D conformer. The presence or absence of the other stems from the clover-leaf structure was not discussed.

High resolution nuclear magnetic resonance (NMR) spectroscopy has made possible the determination of the secondary structure⁹⁻¹⁶, and in favourable cases the tertiary structure of tRNA molecules in solution (ref. 15 and unpublished results of D. R. K., Y. P. W. and S. H. C.). We have already examined the high resolution NMR spectra of the N and D conformers of yeast tRNA_{3^{Leu}} at 220 MHz¹⁶. Two developments prompted us to investigate this problem further: there

has been substantial progress in the interpretation of the low field NMR spectra of tRNA molecules, and the 300 MHz spectrometer now provides much improved spectra.

Pure tRNA_{3^{Leu}} was prepared as reported recently^{17,18}. A solution of yeast tRNA was heated at 60° C for 5 min in the presence of 10 mM MgCl₂ and cooled to 25° C. This 'heat activated' tRNA was subjected to chromatography in two consecutive columns of BD-cellulose, in the presence and absence of magnesium ion. The partially purified tRNA_{3^{Leu}} was converted specifically into its denatured form (tRNA_{3^{Leu}}^D) and purified in a column of Sephadex G-100. Based on ¹⁴C-leucine acceptance and T₁ RNase degradation, it was judged to be more than 90% pure. The tRNA in cacodylate buffer (10⁻² M, pH 7) was denatured by incubation with EDTA (10⁻² M) at 60° C for 5 min. The solution was then concentrated by vacuum dialysis against the cacodylate buffer to 60 mg tRNA ml⁻¹.

Spectra were obtained with a Varian Associates HR 300 NMR spectrometer operating at 300 MHz and were time averaged to improve signal-to-noise. Sample volumes were ~0.12 ml and the temperature was controlled to $\pm 1^\circ$ C. To determine the number of exchangeable protons per tRNA molecule in the low field region of the NMR spectra the integrated intensity in the appropriate spectral region of the tRNA_{3^{Leu}} sample (11-15 p.p.m.) was compared with the integrated intensity of a standard sample of yeast tRNA^{Pha}

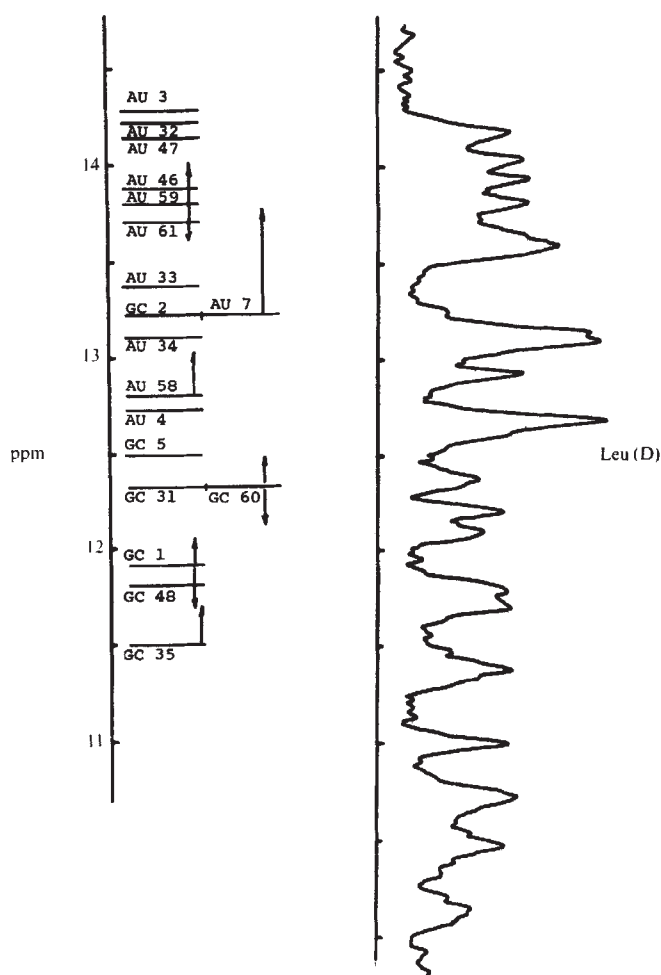


Fig. 1 The 300 MHz proton NMR spectrum of the denatured conformer of yeast tRNA_{3^{Leu}} at 45° C in a solution containing 0.1 M NaCl and 10 mM cacodylate buffer at pH 7. The tRNA concentration was 60 mg ml⁻¹. A stick diagram representation of the NMR spectrum computed for the model shown in Fig. 3 is also presented, together with assignments. The length of one of the smaller horizontal bars represents one resonance.

(1.4 mM) which exhibits 19 resonances per tRNA in the 11 to 15 p.p.m. region^{12,13}. The accuracy of this comparison is good to $\pm 10\%$. Alternatively, we assumed that four peaks located between 14.2 and 13.7 p.p.m. in the NMR spectrum of tRNA_{3^{Leu}}D corresponded to four protons per molecule and then used this to integrate the rest of the spectrum. Both methods gave a value of 18 ± 2 resonances per molecule.

The low field proton NMR spectrum of the denatured form of tRNA_{3^{Leu}}D is shown in Fig. 1. In previous studies we demonstrated that the hydrogen-bonded ring NH protons of Watson-Crick base pairs (AU, A Ψ or GC) give rise to one resonance per base pair in the low field (11 to 15 p.p.m.) region of the NMR spectrum⁹⁻¹⁵. Therefore, integration of the spectrum by the two methods described above indicates that the D conformer contains 18 ± 2 base pairs, and an accurate comparison of the N and D conformer spectra indicates that the D conformer contains ~ 4 less resonances than the N conformer¹⁶. This comparison further indicates that the difference between the N and D conformers cannot be explained in terms of a loss of three base pairs. This is more clearly emphasised by the difference spectrum (Fig. 2) obtained by subtracting the spectrum of the N conformer from that for the D conformer. This suggests that in the change from the N to the D conformer at least five of the original base pairs are lost and at least two base pairs are gained. Since the spectrum of the native conformer can be accounted for accurately in terms of the standard clover-leaf structure (unpublished results of D. R. K., Y. P. W. and S. H. C.) the denatured conformer cannot have a clover-leaf structure. To deduce the probable secondary structure of the D conformer we used a set of semi-empirical rules we have developed for relating the secondary structure of a polynucleotide to the exact location of the low field resonances¹²⁻¹⁴. Resonances from AU and GC base pairs in a double helix are upfield shifted from their intrinsic (unshifted) positions as a result of ring current fields generated by neighbouring bases,

and Table 1 lists a set of parameters which give a satisfactory account of the NMR spectra of other tRNA species, tRNA fragments and model systems. These shift parameters when combined with the unshifted positions (AU $^\circ$ = 14.5 p.p.m., GC $^\circ$ = 13.6 p.p.m. and A Ψ $^\circ$ = 13.5 p.p.m.) enable us to compare the low field NMR spectrum expected for any helical structure^{14,15}.

Table 1 Summary of ring current shift parameters used in calculation of low field proton NMR spectra of tRNA

5'	3'	5'	3'
U = 0	A = 1.1	U = 0	A = 1.1
C = 0	G = 0.6	C = 0	G = 0.7
G = 0	C = 0.1	G = 0	C = 0.2
A = 0.1	U = 0	A = 0	U = 0.1
UA \overline{NV}		CG \overline{SS}	
U = 0.1	A = 0	U = 0.1	A = 0
C = 0.2	G = 0	C = 0.25	G = 0
G = 0.6	C = 0	G = 0.7	C = 0
A = 0.6	U = 0	A = 1.0	U = 0
3'	5'	3'	5'

The notation 5' and 3' refers to the sugar positions at the ends of chains containing the neighbouring bases indicated. The unshifted positions for usual base pairs are: (AU) $^\circ$ = 14.5 p.p.m., (GC) $^\circ$ = 13.6 p.p.m. and (A Ψ) $^\circ$ = 13.5 p.p.m.

Since the D conformer competitively inhibits amino-acylation to the N conformer⁴, some features of the original clover-leaf structure probably remain, and for various reasons we assume that the amino acid acceptor stem is retained (unpublished results of D. R. K., Y. P. W. and S. H. C.). We then examined various models containing one of the following additional structural features: (1) T Ψ C stem + anticodon stem; (2) T Ψ C stem + minor stem; (3) anticodon stem + minor stem. Using the shift parameters of Table 1, NMR spectra were computed for all possible base pairing schemes consistent with these restrictions. The only one which gave a satisfactory fit of the low field spectrum of the D conformer and, perhaps more importantly, the native-denatured difference spectrum, is shown in Fig. 3. The NMR spectrum predicted for this model is shown in Fig. 1 and the calculated (N-D) difference spectrum is compared with the observed difference spectrum in Fig. 2.

The positions of resonances associated with some of the terminal base pairs are sensitive to the stacking of adjacent bases and the range of values expected are also indicated in both Figs 1 and 2 by arrows which show the possible range of shifts. The resonances from AU₇ and AU₅₈ are particularly sensitive to the stacking of the amino acid acceptor stem and the T Ψ C stem, and arrows in Fig. 2 indicate how these two resonances would shift if the two arms were unstacked. Our earlier work with yeast tRNA^{Phe} suggests that AU₇ could be absent from the low field spectrum because of the adjacent GU mismatch^{12,13}. If this is true, we cannot draw any conclusion regarding the relative orientations of the amino acid acceptor stem and the T Ψ C stem. If the AU₇ resonance is present, however, the best fit is obtained when these two stems are stacked.

The model we propose for the D conformer differs from the clover-leaf in that both the DHU stem and the anticodon stem are replaced by a single 'extra' helix which involves pairing between bases of the anticodon loop and the T Ψ C loop in the clover-leaf model. Conversion from the D to the N conformer necessarily involves removing the 'extra' helix and this could account for the large ΔH which is observed experimentally⁶.

The only other data bearing directly on the base pairing structure of the D conformer are the oligonucleotide binding data of Uhlenbeck *et al.*⁵. These experiments show that in the D conformer, the DHU loop and stem regions are quite accessible to base pairing with complementary oligonucleo-

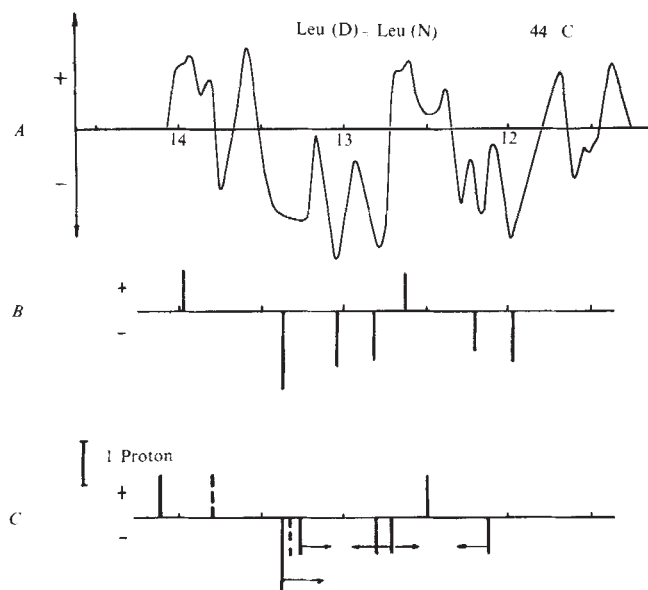


Fig. 2 A, 300 MHz proton NMR difference spectrum obtained by subtracting the spectrum of the native conformer from the spectrum of the denatured conformer of yeast tRNA_{3^{Leu}}. B, A stick diagram representation of the observed D-N difference spectrum. C, The computed D-N difference spectrum based on the assumption that the N conformer has the cloverleaf structure and the D conformer has the structure shown in Fig. 3. Some terminal base pairs may be susceptible to end effects and the expected range for these resonances is also indicated. If the amino acid acceptor stem and the T Ψ C stem are not stacked in the D conformer, there will be additional changes (indicated by dashed lines).

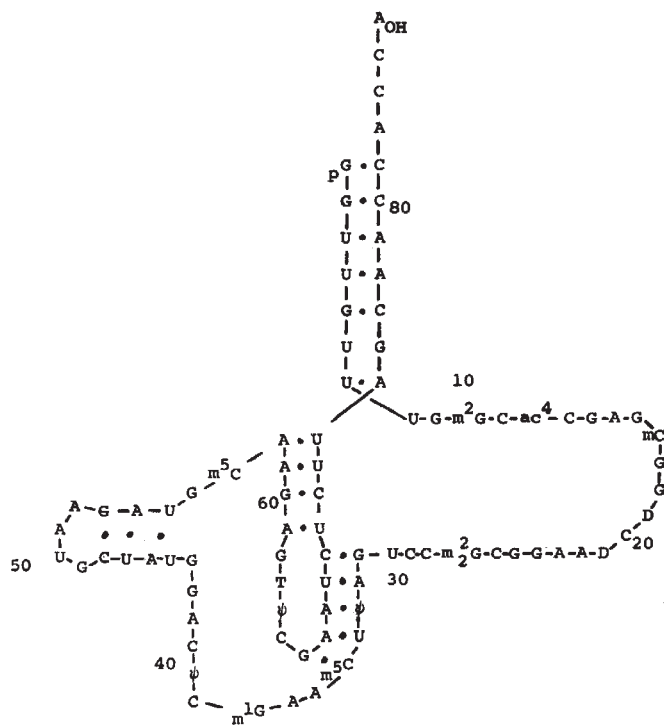


Fig. 3 Proposed model for the secondary structure of the denatured conformer of yeast tRNA^{Leu}.

tides whereas the anticodon loop region which is exposed in the N conformer is 'protected' in the D conformer. These observations are also consistent with the proposed structure. A more complete account in which the results for alternative structures are presented along with more extensive data on both the native and denatured conformers is in preparation.

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DAVID R. KEARNS
YENG P. WONG

Department of Chemistry,
University of California,
Riverside, California 92502

ERIN HAWKINS
SIMON H. CHANG

Department of Chemistry,
Louisiana State University,
Baton Rouge, Louisiana 70821

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Purification of Bacteriophage λ Int Protein

BACTERIOPHAGE λ can direct the formation of small amounts of several λ proteins in a host whose endogenous protein synthesis has been decreased by ultraviolet irradiation before phage infection¹. The phage protein can be labelled by the incorporation of radioactive amino acids after infection. Many of these proteins can be resolved by sodium dodecyl sulphate (SDS) gel electrophoresis and tentative gene identifications can be made by comparing the products of wild type phage infection with those of phage carrying mutation(s) which selectively remove certain gene product(s)². I have purified the *int* gene product using differential label techniques to detect the Int polypeptide. This gene product functions *in vivo* to direct recombination between specific sites in DNA; the radiochemically pure product has been used to investigate the *in vitro* interaction between Int and DNA.

Figure 1 presents a typical double label experiment which confirms the finding^{2,3} of the production of a polypeptide of molecular weight 42,000 dependent on a functional *int* gene. Only this polypeptide is made in significant excess when *int*⁺ phage products are compared with those from an unsuppressed *int* amber (*int* 29) phage. The absence of this polypeptide in *int* 29-infected cells probably does not reflect a polar effect of the amber mutation in the *int* gene on another gene since polypeptide production in this system involves gene N function⁵ and polarity effects of amber mutations are not seen during transcription under the influence of gene N product (personal communication from S. Adhya, M. E. Gottesman and B. de Crombughe, in preparation). That this polypeptide represents the product of the *int* gene and not the product of another gene under *int* gene control can be deduced from the data in Table 1. In this experiment the production of a polypeptide of molecular weight 42,000 in excess of that produced by phage carrying an *int* nonsense mutation is examined for phage carrying either an *int*⁺ gene or one of five different non-suppressible *int* mutations. Four of the five mutant phage yield amounts of this material experimentally indistinguishable from that of wild type, indicating that production of the polypeptide does not depend indirectly on *int* gene function and therefore the polypeptide represents the product of the *int* gene. *int* 41 may represent a promoter mutation, a nonsense mutation responding to a suppressor not tried (*su* 1-5 have been examined), a small deletion or deletion-substitution and so on.

The appearance of differential label of an appropriate mobility on SDS gel electrophoresis can be used as an assay for Int protein. Using this approach I have devised a purification scheme that reproducibly yields a 75% or greater radiochemically pure Int protein. The purification procedure, based on that for T7 endonuclease described by Center and Richardson⁷, avoids the use of detergents or other known denaturants.

Dissociation of protein into individual polypeptide chains is used only in the SDS gel assay. Figure 2a shows the elution profile of radioactivity for the final column step and Fig. 2b shows a typical SDS gel profile of the purest material. 79% of the ^3H -counts recovered from this gel are in a single band corresponding to molecular weight 42,000. In a typical purification procedure, about 10% of the Int detected after the sonication step is recovered from the phosphocellulose column. The molar amount of Int protein can be calculated from its radiochemical activity using an assumed specific activity for leucine in Int (equal to that of the labelling medium) and an assumed leucine content for Int (35 mol leucine mol $^{-1}$ Int protein). Such a

calculation indicates the formation of about 50 ng Int product per 200 ml of infected ultraviolet-irradiated cells. At least twenty times more Int can be recovered by applying the identical purification scheme to unirradiated cells which have produced Int after thermal induction of a λ prophage (data not shown).

Table 1 Int Protein Production by Int Mutants in Ultraviolet-irradiated Cells

Phage	% Incorporation into Int *
$\lambda b2int^+ c1857$	3.98
$\lambda b2int21 c1857$	3.83
$\lambda b2int44 c1857$	3.39
$\lambda b2int4 c1857$	3.33
$\lambda b2int6 c1857$	2.12
$\lambda b2int41 c1857$	0.01

The proteins made after infection by each phage listed were labelled with ^3H -leucine. In each case, a control culture, infected with $\lambda b2int29 c1857$, was labelled with ^{14}C -leucine. Growth of M159, irradiation, infection, labelling, sonication and electrophoresis were as in Fig. 1. The *int* mutations used were isolated by Gottesman and Yarmolinsky⁶.

* % Incorporation into Int is (^3H c.p.m. at molecular weight 42,000/total ^3H c.p.m. recovered from the gel) - (^{14}C c.p.m. at molecular weight 42,000/total ^{14}C c.p.m. recovered from the gel) $\times 100$.

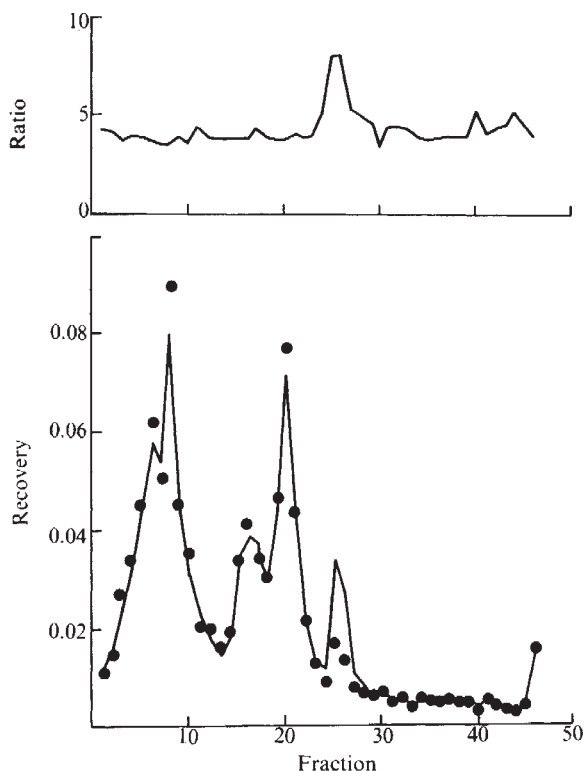


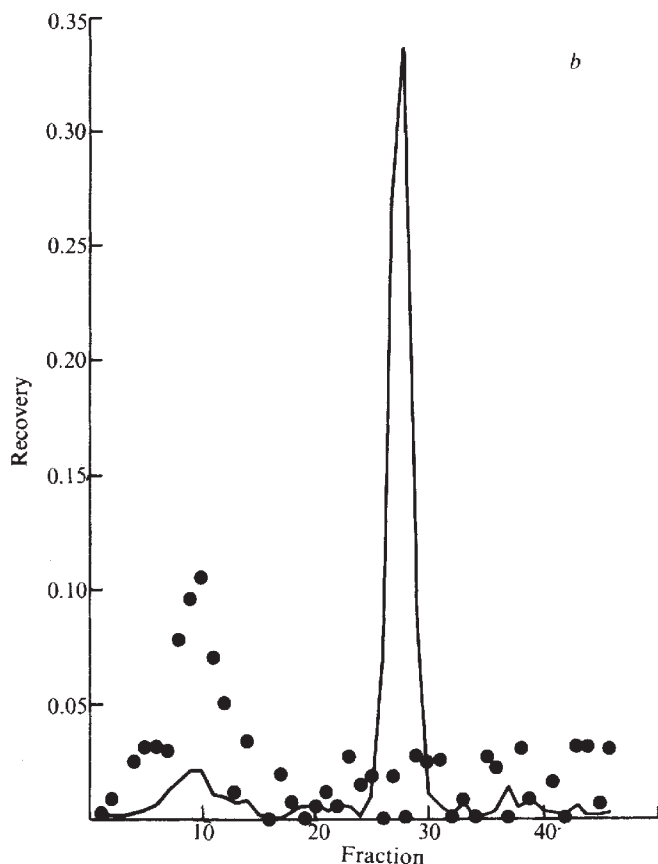
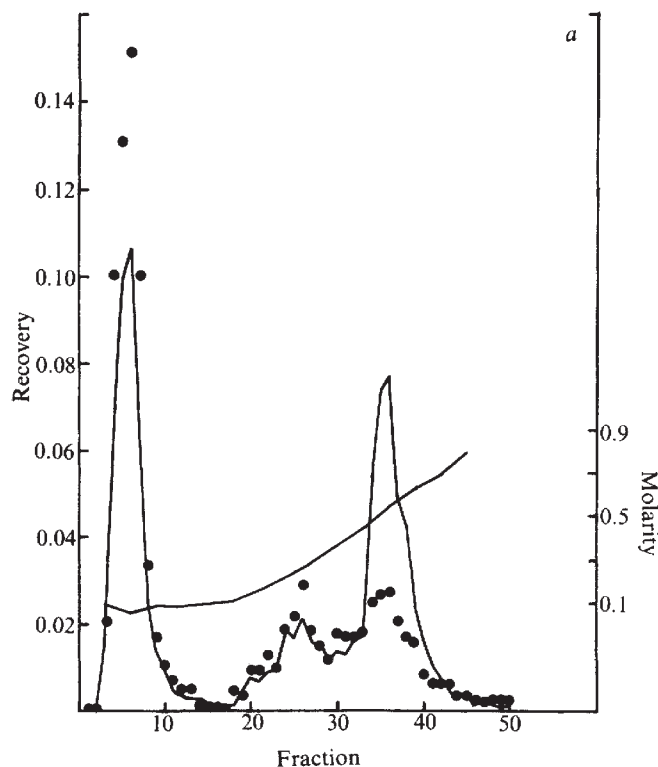
Fig. 1 SDS gel electrophoresis of protein made in ultraviolet-irradiated hosts. *E. coli* strain M159 UV s , *su* $^-$ was grown on 400 ml medium A with 0.4% maltose at 38°C. At mid-log phase the cells were chilled and irradiated with ultraviolet light (8 min at 25 cm from a 15 W GE germicidal lamp). MgSO_4 was added to 0.02 M, the culture was divided and each half was infected with a multiplicity of infection = 3 of $\lambda b2 c1857$ or $\lambda b2 int 29 c1857$. After 5 min adsorption at 38°C, 2.0 mCi 4-5- ^3H -leucine (2.6 Ci mmol $^{-1}$) or 0.2 mCi U- ^{14}C -leucine (261 mCi mmol $^{-1}$) were added to each half. After 60 min of slow shaking at 38°C the cells were chilled, spun, washed twice in 0.02 M Tris-HCl, pH 7.4, mixed with a ten-fold excess of untreated cells in the same buffer, disrupted by sonication (four bursts of 5 min with 5 min between bursts, temperature $< 10^\circ\text{C}$), and spun at 10,000g for 15 min. A sample was made 1% in SDS, 1% β -mercaptoethanol, and 6% sucrose and run in a 7% polyacrylamide gel with SDS according to the procedure of Weber and Osborn⁴. A 7.5 cm gel was cut into forty-six equal fractions, dissolved overnight in 0.5 ml 30% H_2O_2 at 50°C, suspended in a scintillation cocktail containing toluene, 0.85% Beckman TLA, and 16.6% Beckman Biosolv BBS3, and counted. The ratio of c.p.m. in each fraction to total c.p.m. for all fractions is plotted on the lower ordinate as a continuous line for ^3H and as discrete points for ^{14}C . The ratio of ^3H c.p.m. to ^{14}C c.p.m. for each fraction is plotted on the upper ordinate. 13,530 c.p.m. and 3,300 c.p.m. of ^3H and ^{14}C respectively, were recovered from the gel. Direction of electrophoresis is from left to right. Standards of chymotrypsinogen, ovalbumin, catalase and β -galactosidase were run in parallel gels for molecular weight calibration. These proteins (of molecular weight 26,000, 43,000, 60,000 and 130,000) migrate to positions corresponding to fractions 13, 20, 27, 33 and 40, respectively.

Although the material of Fig. 2b is nearly homogeneous in radioactive label, it contains greater than 1,000-fold excess of unlabelled protein, none of which is Int protein (or any other λ protein.) Nevertheless, the sedimentation behaviour of this crude Int protein in the presence and absence of its putative DNA substrate can be studied by observing the radiochemical label.

By comparison with marker proteins of known sedimentation coefficient, Int has $S_{20,w} = 3.75$ in sucrose gradients containing 0.05 or 0.2 M potassium phosphate buffer. The sedimentation coefficient is characteristic of globular proteins of molecular weight about 50,000.

If Int is mixed with λ DNA before layering on the gradient, the sedimentation behaviour changes markedly. Most of the Int protein sediments with the DNA at $S_{20,w} = 30$ with a smaller amount of Int protein sedimenting near the top of the gradient at the 3-4S value seen for Int alone. The binding of Int to DNA becomes progressively weaker as the ionic strength increases and is undetectable in gradients containing 0.3 M potassium phosphate, indicating an electrostatic component of the binding reaction. Under all conditions, the binding is destroyed by prior heating of Int to 65°C for 15 min. For a given amount of Int protein, the proportion of Int bound to DNA increases with increasing amounts of DNA added to the incubation mixture. An indication of the strength of binding can be obtained from the molar concentrations of Int protein and DNA that yield half maximal binding. In 0.05 M potassium phosphate buffer, half binding of 1×10^{-10} mol l $^{-1}$ Int is seen at 1×10^{-10} mol l $^{-1}$ DNA. This apparently tight binding has been examined using DNAs with different attachment site composition in an attempt to discover a component of binding specific for the putative substrate of Int. Over a wide range of ionic strengths, DNA concentrations, divalent cation, ATP and SAM concentrations, no significant difference was seen for Int binding to phage DNA with 0, 1 or 2 attachment sites per genome ($\lambda b538$, λ , and λatt^2 , respectively^{8,9}). Indeed, Int seems to bind as well to phage T4 and phage T3 as well as that of phage λ , indicating that the binding is not specific for a nucleotide sequence unique to λ nor for a pattern of methylated bases.

The tight but non-specific binding of Int to DNA must be reconciled with the highly specific recombination event which is sponsored by Int. Several possibilities are that: (a) Int is not the site-specific element in integration and does not show



specific interaction with attachment sites *in vivo* or *in vitro*, (b) the specific binding of Int has been lost during purification either through denaturation or removal of accessory proteins or factors, or (c) Int, as purified, does possess specific binding, but this is masked experimentally by a large non-specific component. With regard to the latter possibility, the binding

Fig. 2 *a*, Phosphocellulose column elution profile of partially purified radiochemically labelled Int protein. To 147 ml of the sonic extract described in Fig. 1, 24.5 ml of 5% streptomycin sulphate was added. After stirring, the mixture was spun and the pellet resuspended in 23.5 ml 0.05 Tris-HCl, pH 8. After this, all solutions were 0.01 M in β -mercaptoethanol unless otherwise stated. The resuspended streptomycin pellet was fractionated with ammonium sulphate; the material precipitating between 40 and 70% saturation was resuspended in 2 ml 0.02 M potassium phosphate buffer, pH 7.5, and dialysed overnight against 0.01 M potassium phosphate buffer, pH 7.5. A 3 ml sample of the dialysed material was loaded onto a 40 ml column of Whatman DE52 equilibrated with the same buffer and was immediately eluted with 0.2 M potassium phosphate buffer, pH 7.4. A fraction representing the peak of total protein, with an A_{280}/A_{260} ratio greater than 0.75 was loaded onto a 3 ml column of Whatman P11 phosphocellulose equilibrated with 0.1 M potassium phosphate buffer, pH 7.4. The column was washed with one bed volume of this buffer and a linear gradient of potassium phosphate buffer, pH 7.4, was used for elution. Samples (1 ml) were collected and aliquots were taken for radioactivity and conductivity. The ratio of c.p.m. in each fraction to total c.p.m. for all fractions is plotted on the left ordinate as a continuous line for ^3H and as discrete points for ^{14}C . Molarity of phosphate buffer in each fraction is plotted on the right ordinate. *b*, SDS gel electrophoresis of Int protein purified from ultraviolet-irradiated cells. Fractions 34–38 of the phosphocellulose column elution were pooled. A sample was made 1% SDS, 1% β -mercaptoethanol, heated to 55°C for 30 min, then dialysed against 0.01 M β -mercaptoethanol, 0.1% SDS and 0.01 M sodium phosphate buffer, pH 7.4, overnight. SDS gel electrophoresis and subsequent analysis were performed as in Fig. 1. 948 c.p.m. and 76 c.p.m. of ^3H and ^{14}C were recovered from the gel.

of Int to DNA may be similar to the binding pattern of RNA polymerase to T7 DNA and of cyclic AMP receptor protein to *gal* or *lac* DNA. In both of these cases, highly purified, highly active protein preparations which exhibit specific action on certain DNA sites *in vivo* and *in vitro* show tight but predominantly non-specific binding to DNA. Specific binding of the receptor protein to catabolite-sensitive sites has not yet been discerned¹⁰; in the case of RNA polymerase, binding which reflects the *in vivo* specificity for promoter sites has been revealed after an extensive search for the binding variables¹¹.

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HOWARD A. NASH

Laboratory of Neurochemistry,
National Institute of Mental Health,
Bethesda, Maryland 20014

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Acid Protease Activity in *Plasmodium falciparum* and *P. knowlesi* and Ghosts of Their Respective Host Red Cells

ERYTHROCYTIC stages of the malarial parasite apparently obtain the bulk of their amino acids from digestion of host cell haemoglobin¹⁻⁵. In addition, intracellular degradation of unnecessary organelles occurs within the parasite following invasion of the host cell. Thus, it is rather surprising that there have been relatively few studies on the proteolytic enzymes of *Plasmodium*, since these would presumably be involved in both processes. Cell-free extracts of *P. gallinaceum* degraded denatured globin at pH 6.5, while haemoglobin was digested very slowly⁶. Data of Cook *et al.*⁷ indicate the existence of two proteinases, with pH optima of 4 and 8 (*P. berghei*) and 5 and 8 (*P. knowlesi*). In each case, the alkaline protease was the more active.

We have found that the specific activity of an acid protease in *P. berghei*-infected red cells is five to ten times that of uninfected cells⁸. The enzyme degrades haemoglobin optimally at pH 2.5 to 3, and there is little activity in the alkaline range. Its properties are very similar to those of a protease from host cell ghosts.

In this report we describe some properties of proteases from *P. falciparum*, *P. knowlesi*, and the red cell ghosts of their corresponding hosts, Aotus and Rhesus monkeys, respectively. Our data indicate: (1) each species of parasite contains a protease that degrades haemoglobin optimally at pH 3.6, and which differs in pH response and sensitivity to inhibitors from that of host cell ghosts. (2) The enzymes from the two species of monkeys differ from each other in sensitivity to several

inhibitors and in pH response. (3) The acid proteases from both ghosts and parasites are extremely sensitive to pepstatin, an inhibitor of proteases such as cathepsin D and are also very sensitive to chymostatin, an inhibitor of chymotrypsin-like proteases.

Most procedures used for obtaining parasites give preparations that are somewhat contaminated with membranes and other materials of the host cell⁹⁻¹⁰. Since host cell proteases are tightly bound to the red cell stroma¹¹, the parasites could be contaminated with varying amounts of host cell enzyme. We therefore decided to initially study the distribution of proteinase activity between the ghost and parasite-rich fraction, and then to compare the properties of the enzymes from each source.

Red cells were freed of leukocytes by a modification of the procedure of Martin¹², as described elsewhere⁸. In most preparations, well over 90% of the leukocytes were removed. Red cells were then haemolysed in a solution of 0.01 M potassium phosphate, pH 6.8, containing 2×10^{-5} M EDTA (PE). The sample was centrifuged for 5 min at 17,500g. In control cells, the haemolysate was decanted from the ghosts. The latter were suspended in PE, and both fractions were recentrifuged. The ghosts were washed at least twice in this manner, with the washing fluids being added to the haemolysate. Samples were kept frozen until use.

In infected cells, the haemolysate was decanted from the ghosts and parasites. The ghosts, which formed a clear pellet over the parasites, were separated from the latter. The ghosts and parasites were suspended in PE, all three fractions were recentrifuged. A small amount of ghost material was recovered from the haemolysate and from the parasites. This was pooled

Table 1 Specific Activity and Intracellular Distribution of Acid Proteinase and Acid Phosphate in Normal and Infected Red Cells of Monkeys

	<i>P. knowlesi</i> in Rhesus monkey			<i>P. falciparum</i> in Aotus monkey			Experiment 2
	Controls	Infected sample 1	Infected sample 2	Controls	Infected sample 1	Infected sample 2	
Specific activity							
Proteinase, total	8.9 (13)	24.4 (28)	27.6 (33)	7.8 (5.5)	10 (6.7)	10 (12)	14.6 (16)
Haemolysate	6.4	11	11	2.9	3.2	5.4	3.4
Ghosts	86.5	180	180	126	70	61	105
Parasites	—	2,600	2,400	—	630	570	1,150
Acid phosphatase, total	0.583	0.438	0.44	0.15	0.30	0.27	—
Haemolysate	0.609	0.24	0.31	0.17	0.30	0.23	—
Ghosts	1.50	0.41	0.49	0.20	0.25	0.36	—
Parasites	—	2.4	1.1	—	1.8	0.48	—
Distribution							
Proteinase, Haemolysate	42	34	32	50	45	43	15
Ghosts	58	33	29	50	29	11	15
Parasites	—	33	39	—	25	45	71
Acid phosphatase, Haemolysate	81	87.5	90	97	96	95	—
Ghosts	19	9.0	8	3	2.3	3.4	—
Parasites	—	3.5	2	—	1.6	1.3	—
Protein, Haemolysate	91	88.4	94.0	97.8	96.7	97.0	96.9
Ghosts	9	11.2	5.4	2.2	2.3	2.8	2.2
Parasites	—	0.35	0.5	—	1.0	0.27	0.95

P. knowlesi in Rhesus monkeys and *P. falciparum* in Aotus monkeys were maintained as described previously⁸. Rhesus blood was collected when the parasites were primarily in the ring (40%) and early trophozoite (40%) forms (sample 1) or in the early trophozoite (42%) and late trophozoite (42%) forms (sample 2). The parasitaemia was 30% in sample 1 and had fallen to 16% by sample 2. Blood from Aotus monkeys was removed from the animal when 92% of the parasites were in the ring form (sample 1) and the parasitised red cells were maintained in an *in vitro* culture system^{5,13}. Sample 2 was taken when 66% of the parasites were in the trophozoite stage. The initial parasitaemia was 37%. In experiment two (last column), blood was taken when the parasites were in the ring (44%) and trophozoite (40%) stages (parasitaemia=40%). Proteinase activity was tested at pH 3.0, as described elsewhere⁸, using haemoglobin as substrate. Acid denaturation of haemoglobin did not increase the rate of breakdown. Results are expressed as μ g of bovine serum albumin equivalents released per h per mg protein. Acid phosphatase was measured at pH 4.5 by the method of Torriani¹⁴, using *p*-nitrophenylphosphate as substrate. Data are expressed as μ moles *p*-nitrophenol released per h per mg protein. Protein was determined by the method of Lowry *et al.*¹⁵. Distributions of enzymes and protein are given as the percentage of the total recovered that was found in a given fraction. Values in parentheses denote the specific activity as calculated from the sum of the various fractions. The percentage of reticulocytes in the blood of Rhesus monkeys was 0.9%. In another experiment, not shown, it was 0.6% and 0.7% in samples 1 and 2, respectively. In the latter experiment, 39% and 58% of the proteinase was recovered in the parasite fraction at the two sampling times. In the Aotus monkey, the reticulocyte count was 1.2% and 0.6% for the two samples in Experiment 1, and 1.2% in Experiment 2.

with the remainder of the ghost fraction. The wash fluids from the ghosts and parasites were added to the haemolysate. The first centrifugations of the ghost fraction yielded additional parasites, and this process was repeated until a parasite-free ghost fraction was obtained.

The specific activity of acid protease of infected Rhesus red cells was about 2.5 times that of normal cells (Table 1). The parasite-rich fraction was greatly enriched in acid proteinase, the specific activity being about 100 times that of the infected cells. About 35% to 40% of the enzyme, and 0.5% or less of the cell protein was associated with the parasites. In a second experiment (not shown), 39% and 58% of the proteinase appeared in the parasite fraction in the two stages. There was little difference in specific activity between stage 1, which contained 40% ring stages and 40% early trophozoites, and stage 2 which contained 47% early trophozoites and 42% late trophozoites.

Acid phosphatase, which is often present in the same sub-cellular compartment as acid proteinase, was found primarily in the haemolysate, with less than 5% appearing in the parasite fraction. The specific activity was similar in infected and non-infected cells.

The Aotus-*P. falciparum* system differed somewhat from the *P. knowlesi*-infected red cells in that there was little difference in specific activity between normal and infected cells. Also, there seemed to be a greater variability in the distribution of proteases, 71% of the enzyme being localised in the parasites in one case. The difference could be due to leakage of enzyme, as the parasitaemia was similar in the two experiments. Again, very little of the protein of the infected cells was present in this fraction, so that there was a great enrichment of the proteinase in the parasites.

To determine if the parasites and the host cells contain distinct proteases, the pH response and the susceptibility to inhibitors of the enzyme from each source were tested. Maximal activity for the *P. knowlesi*-enzyme occurred at pH 3.6. There was considerable activity between pH 3.0 and 4, and almost none at pH 2 (Fig. 1). In contrast, the host cell exhibited maximal activity at pH 2 to 2.5, and was relatively inactive at pH 3.5 and above. The parasites also had a reasonably active alkaline protease. Ghosts from infected cells show a pH response similar to that of ghosts from normal cells.

Maximal protease activity in *P. falciparum* also occurred at pH 3.6, while that of the enzyme from host cell ghosts occurred at pH 2.5. In this case, however, there was little difference in

activity between pH 2.5 and 4 in both ghosts and parasites. Again, there was some activity in the alkaline range.

A number of specific and very potent proteinase inhibitors have been isolated from cultures of actinomycetes¹⁶⁻¹⁹, and these have been of value in characterising proteases of various mammalian cells²⁰⁻²². Thus, pepstatin inhibits cathepsin D in submicrogramme quantities, leupeptin and chymostatin are potent inhibitors of cathepsin B, while antipain is most active against cathepsin A¹⁸. These compounds are also potent inhibitors of various extracellular proteases.

Table 2 Concentration of Inhibitors Giving 50% Inhibition of Acid Proteinase

	<i>P. knowlesi</i>	Rhesus ghosts	<i>P. falciparum</i> ($\mu\text{g ml}^{-1}$)	Aotus ghosts
Pepstatin *	0.010	<0.0008	0.002	<0.002
Chymostatin	0.10	~0.005	0.020	~0.0025
Antipain	5.0	~0.5	1.0	~0.20
Leupeptin	~30	~5.0	2.5	~0.3

* The concentrations indicated for pepstatin on the ghosts were the lowest tested and produced more than 95% inhibition.

Pepstatin was the most potent inhibitor against the protease of both species of parasites and ghosts (Figs 2, 3, Table 2). Complete inhibition of the protease from red cell ghosts from Rhesus and Aotus monkeys occurred at 0.8 and 2 ng ml, respectively, the lowest concentrations tested. The parasite proteases were also very sensitive to pepstatin, 50% inhibition occurring at 10 ng ml⁻¹ and 2 ng ml⁻¹, respectively, for *P. knowlesi* and *P. falciparum*. A small percentage of the activity in each species was insensitive to the inhibitor.

Chymostatin also was a potent inhibitor of protease activity in both ghosts and parasites. In both species of ghosts, there was a rapid transition from complete to no inhibition, and 50% inhibition occurred at about 5 and 2.5 ng ml⁻¹ for the enzyme from Rhesus and Aotus ghosts, respectively (Table 2). The enzymes from the parasites were inhibited at somewhat higher concentrations, and the change in degree of inhibition was more gradual than in the ghosts.

Antipain and leupeptin also inhibited protease activity, but at somewhat higher concentrations than pepstatin and chymostatin. Again, the transition from complete to no inhibition was very sharp in the ghosts and more gradual in the parasite, and the enzymes from the ghosts were the more sensitive.

The inhibition of the parasite enzyme was similar at pH 3 and 3.9 (Fig. 2), and tripling the amount of enzyme did not affect the degree of inhibition. None of these compounds inhibited the alkaline protease (Table 3) at the highest concentrations tested.

The acid proteinase of the parasites was not greatly affected by iodoacetamide or *p*-chloromercuribenzoate (Table 3). The insensitivity to these compounds, and lack of activation by dithiothreitol suggest that the major enzyme lacks essential sulphhydryl groups. Suramin, an antitrypanosomal drug that accumulates in lysosomes and inhibits proteolysis within these particles²³ also was without effect, as was phenylmethane-sulphonyl fluoride, an inhibitor of serine proteases. None of these compounds had any effect on the alkaline protease, except perhaps high concentration of dithiothreitol.

These data indicate that both *P. knowlesi* and *P. falciparum* have an acid proteinase that degrades haemoglobin optimally at a pH of 3-3.6. We have found that *P. berghei* contains a similar enzyme, with optimal activity at pH 2.5 to 3 (ref. 8). The existence of an active acid proteinase in the malarial parasite had not been previously reported. Cook *et al.*⁷, using denatured globin as substrate, found that *P. berghei* and *P. knowlesi* contained an acid proteinase that acted optimally at

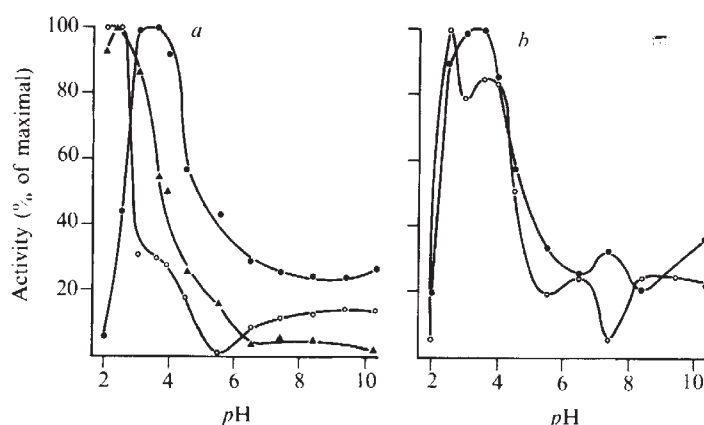


Fig. 1 pH response of proteases from parasites and host cell ghosts. *a*, Rhesus monkey; $\circ$, RBC ghosts; $\blacktriangle$, ghosts of infected cells. $\bullet$, *P. knowlesi*. *b*, $\circ$, RBC ghosts, Aotus monkey; $\bullet$, *P. falciparum*, FVO strain. Buffers: Glycine-HCl, pH 2-3; NaAcetate-acetic acid, pH 3.6-5.5; potassium phosphate, pH 6.5-7.4; Borate-NaOH, pH 8.4-10.3. Specific activities: *P. knowlesi*, 2560; Rhesus ghosts, 184; Rhesus ghosts from infected cells, 340; *P. falciparum*, 2210; Aotus ghosts, 164.

pH 4 and 5, respectively. But the maximal activity in both species occurred at pH 8, and the acid proteinase lost most of its activity within 12 h at 2° C. We have found that haemoglobin is degraded much more slowly in the alkaline range in both species studied here, and that *P. berghei* exhibits almost no activity towards haemoglobin above pH 6.5⁸. We have also found the acid proteinase to be relatively stable. Further evidence that our enzyme differs from that of Cook *et al.* is the sensitivity of their enzyme to EDTA.

The data here also differ in certain respects from our findings on the *P. berghei*-mouse system⁸. In the latter, the specific proteinase activity of infected cells is five to ten times that of the normal cells. Interestingly, the specific activity of normal mouse red cells is about fifteen-fold higher than that of either monkey, while the activity of infected cells is thirty to seventy-five times that of the infected primate cells. The distribution of acid phosphatase between the haemolysate, ghosts and parasites differs greatly from that of the acid proteinase, and suggests that the parasites have little of the phosphatase. Such a conclusion would be consistent with results of a recent cytochemical study, in which bodies positive for acid phosphatase were found in the host cell but not the parasite²⁴. Our procedure for obtaining parasites could, however, lead to leakage of enzyme.

The pH response curves indicate that both *P. knowlesi* and *P. falciparum* contain an acid proteinase that is distinct from that of the host red cell. This is especially clear in the Rhesus-*knowlesi* system, where the enzyme from the host cell has a very low pH optimum. It is of interest that the pH response of the two species of monkeys differs, the Aotus enzyme exhibiting very little activity at pH 2, while the Rhesus enzyme is fully active at this pH.

Recent studies have shown that some enzymes attributed to the parasite may be of reticulocyte origin²⁵. But it seems unlikely that the 'parasite' proteases described here are due to contamination by reticulocytes. In the case of the *P. knowlesi*-Rhesus monkey system, the percentage of reticulocytes (Table 1) was 0.9%. In a second experiment, the percentages for samples 1 and 2 were 0.6% and 0.7% respectively. In this experiment, 39% and 58% of the acid protease appeared in the 'parasite' fraction. In the experiments on *P. falciparum* the percentage of reticulocytes ranged from 0.6% to 1.2%, and there was no correlation between the reticulocyte level and the percentage of enzyme appearing in the parasite fraction. It may also be noted that the per cent of both total protein and acid phosphatase that appears in the parasite fraction is extremely low.

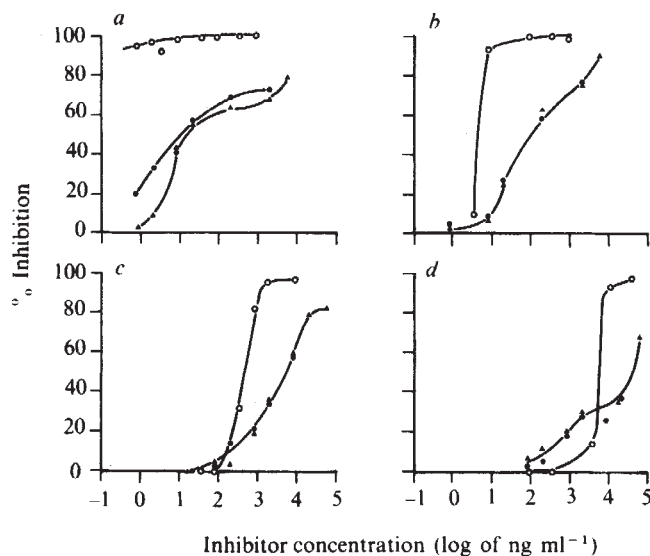


Fig. 2 Effects of inhibitors on acid proteases of *P. knowlesi* and ghosts of Rhesus red cells. a, Pepstatin; b, chymostatin; c, antipain; d, leupeptin. ○, Rhesus red cell ghosts, tested at pH 2.5; ●, *P. knowlesi*, tested at pH 3.0; ▲, *P. knowlesi*, tested at pH 3.9.

The pH response of the protease from the ghosts and parasites also argues against a possible reticulocyte origin (Fig. 1). Thus, at pH 2.0, the enzyme from Rhesus red cells is fully active, while the *P. knowlesi* enzyme has virtually no activity. For the 'parasite' enzyme to be from reticulocytes, it would not only be necessary for the reticulocytes to be completely devoid of the erythrocyte protease, but it would also imply that the erythrocytes are capable of synthesising a new enzyme that is distinct from that found in reticulocytes.

The results with the inhibitors from actinomycetes are of interest and also suggest that the parasites and red cell ghosts contain distinct enzymes. All of the inhibitors are more effective against the parasite protease, the concentration giving 50% inhibition usually differing by an order of magnitude or more. The sensitivity to pepstatin and chymostatin of the ghosts enzyme appear to be the greatest yet reported, as

Table 3 Effects of Various Compounds on Protease Activity of Red Cell Ghosts and Parasites

		<i>P. knowlesi</i>		Activity (% of control)			Aotus ghosts pH 10.3
		pH 3.9	pH 10.3	Rhesus ghosts pH 2.5	<i>P. falciparum</i> pH 3.0	<i>P. falciparum</i> pH 10.3	
Phenylmethanesulphonylfluoride	0.5 mM	103	90	90	92	97	105
	1.5 mM	—	90	—	81	98	97
Iodoacetamide	1.0 mM	90	—	—	—	—	—
	2.0 mM	—	90	96	101	102	99
	5.0 mM	—	96	95	98	113	98
<i>p</i> -Chloromercurobenzoate	0.5 mM	84	94	97	90	105	—
	1.0 mM	—	90	94	81	106	106
EDTA	2.0 mM	95	91	100	116	105	—
	10 mM	—	91	—	103	107	93
Dithiothreitol	1.0 mM	108	116	100	99	104	93
	5.0 mM	—	61	—	92	105	74
Suramin	0.5 mM	90	—	—	—	—	—
Pepstatin	2 µg ml ⁻¹	—	96*	—	—	—	—
Chymostatin	2 µg ml ⁻¹	—	96*	—	—	—	—
Antipain	8 µg ml ⁻¹	—	107*	—	—	—	—
Leupeptin	20 µg ml	—	109*	—	—	—	—

* Tested at pH 9.3.

nanogramme quantities cause complete inhibition (recently we have found the sensitivity to these inhibitors of partially purified enzyme from *P. berghei* to be even greater than the enzymes reported here). The molar ratio of enzyme to inhibitor is, however, important in determining the sensitivity².

In view of their reported specificity^{18,21}, it is surprising that each of the inhibitors is capable of inhibiting virtually all the protease activity. Thus, while pepstatin inhibits acid proteases at very low concentrations, it reportedly does not inhibit any of the proteases inhibited by chymostatin (chymotrypsin, cathepsin B), antipain (trypsin, papain, cathepsin A), or leupeptin (papain, cathepsin B)¹⁶⁻²⁰. Similarly, the other three inhibitors reportedly do not inhibit acid proteases, except at concentrations much greater than those used here. Thus, the red cells and parasites each contain a protease that is unusual in being susceptible to all of the inhibitors.

Our data suggest that the major acid proteinase of both the parasites and the ghosts is like cathepsin D. This is based on the pH optima, the great sensitivity to pepstatin, and the lack of sensitivity to the sulphhydryl reagents or the serine protease inhibitor (PMSF) tested in Table 3.

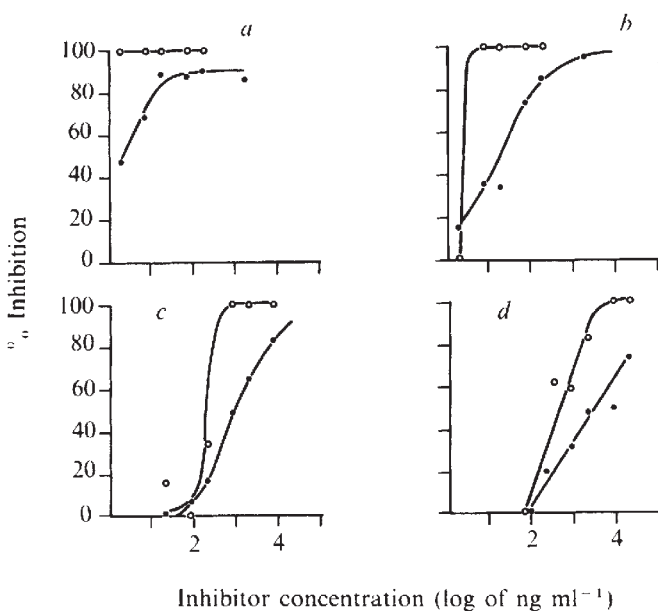


Fig. 3 Effects of inhibitors on acid proteases of *P. falciparum* and ghosts of *Aotus* red cells. *a*, Pepstatin; *b*, chymostatin; *c*, antipain; *d*, leupeptin. ○, *Aotus* red cell ghosts, tested at pH 2.5; ●, *P. falciparum*, FUP strain, tested at pH 3.0.

It will be of interest to determine the susceptibility to the malarial proteases of haemoglobins of various species. *P. falciparum* can infect *Aotus* monkeys, which contain a human type haemoglobin²⁶, but cannot invade *Macacus* monkeys, which do not²⁷. The ability of a given species of parasite to infect a particular species of organism could perhaps be in part determined by its ability to degrade the haemoglobin of the host red cell. The marked difference in the pH-response curve of the enzyme from the red cell ghosts of *Aotus* and Rhesus monkeys is of interest in this respect.

Finally, we should like to suggest that protease inhibitors may be of value in inhibiting the growth of the malarial parasite by selectively acting on a process that is essential to the parasite but not, at least over a short period, to the cells of the host. The erythrocytic stages of the parasite apparently must obtain nutrients by degradation of host cell haemoglobin, and also apparently must degrade many of its own organelles. Therefore, it seems to be very dependent upon the action of

intracellular proteases. Protease inhibitors would perhaps be more useful than drugs that inhibit macromolecular synthesis in all eukaryotic cells since the former are directed specifically at a metabolic difference between the host and the invading cells. In this respect, preliminary experiments show at least one of the protease inhibitors, pepstatin, greatly inhibits the growth of the parasite in an *in vitro* culture system.

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MICHAEL R. LEVY

Department of Biology,
Southern Illinois University,
Edwardsville, Illinois 62025

W. A. SIDDIQUI

Department of Tropical Medicine
and Medical Microbiology,
University of Hawaii

S. C. CHOU

Department of Pharmacology,
University of Hawaii School of Medicine,
Honolulu, Hawaii 96816

Received July 19; revised November 26, 1973.

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Prostaglandin E₂ as stimulator of haemopoietic stem cell proliferation

ALTHOUGH a strict correlation between prostaglandins and the cyclic AMP system has been suggested for several cell types, and the mitogenic effect of cyclic AMP has also been proved^{1,2}, the mitotic effect of prostaglandins has been demonstrated only for some cell systems³. Also, little is known about the haemopoietic effect of prostaglandins. As proved by Dukes⁴, several prostaglandins, especially PGE₂, enhance the ⁵⁹Fe and ¹⁴C-glucosamine incorporation into bone marrow cells and potentiate the effect of erythropoietin, *in vitro*. We have studied the effect of PGE₂ on the proliferative state of haemopoietic stem cells (spleen colony forming units:CFU).

In the normal steady state by far the greater part of the pluripotent stem cell population is either not in cell cycle (G₀ state⁵) or is passing through a rather long cell cycle. Stem cells, however, may be triggered into the cell cycle by imposed stimuli for differentiation or regeneration *in vivo* or by certain drugs such as steroids¹ *in vitro*.

The proliferative state of CFU can be well characterised by their *in vitro* sensitivity to ³H-thymidine of high specific activity⁶. In the normal steady state less than 10% of the CFU population is sterilised by 30 min exposure to ³H-thymidine.

The test system used was almost identical with that described by Byron⁷. Bone marrow was aspirated from the femora of 12–14 weeks old Balb/c × CBA/F₁ hybrid male mice and suspended in Fischer's medium (Gibco, New York). One millilitre of this bone marrow suspension (containing 3.1×10^6 – 4.2×10^6 nucleated cells) was incubated in siliconised bottles, in an atmosphere containing 5% CO₂, at 37° C in a shaking water bath. Various concentrations of

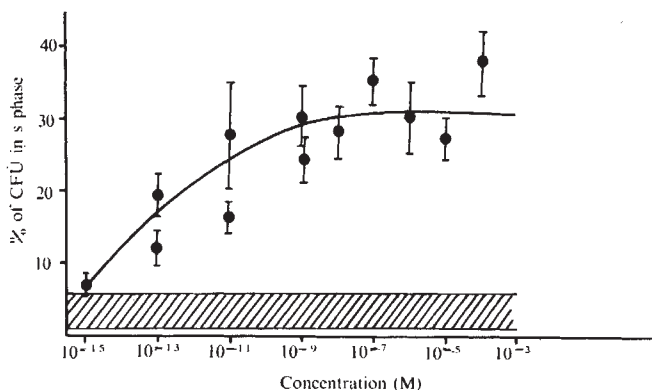


FIG. 1 Effect of PGE₂ on the percentage of CFU in S phase, *in vitro* measured as the percentage of CFU sterilised by 30 min exposure to ³H-thymidine. Each point represents the mean and standard deviation of one experiment. Hatched over control values.

PGE₂ (Chinoin, Budapest) were added to the cultures at the beginning of the incubation. The stock solution contained 2–5,000 µg ml⁻¹ in 96% ethanol. PGE₂ dilutions were always freshly prepared in Fischer's medium and added to the cultures in a volume of 0.1 ml. The ethanol concentration of the cultures never exceeded a final concentration of 0.05%. The same quantity of ethanol was added to the control samples. The number of CFU in S phase was measured according to Becker *et al.*⁸. After incubation with various concentrations of PGE₂ for 2.5 h, 200 µCi per 0.2 ml of pre-warmed, isotonic ³H-thymidine (21–28 Ci mmol⁻¹, Radiochemical Centre, Amersham), was added to half of the cultures. After 30 min incubation with ³H-thymidine the bone marrow suspensions were diluted with ice-cold Hanks' solution to the required cell concentration and were transplanted immedi-

ately into 10–10 lethally (850 R ⁶⁰Co) irradiated recipients. The spleen colony count was determined 9 d after transplantation⁷. The difference in CFU count between cultures incubated with or without ³H-thymidine was a measure of the number of CFU in S phase.

The effect of PGE₂ on CFU proliferation was studied in the dose range 10⁻⁴–10⁻¹⁵ M (Fig. 1). A stimulatory effect could be clearly demonstrated at 10⁻¹³ M and reached its plateau at 10⁻⁹ M. No cytotoxic effect was found even at the highest concentration studied.

Since it has been proved that cyclic AMP may trigger the entry of CFU into the cell cycle *in vitro*⁴ and prostaglandins may enhance cyclic AMP production in certain cells^{2,8,9}, we next studied whether the PG effect could be inhibited by decreasing the intracellular cyclic AMP level. Imidazole increases the enzymatic destruction of cyclic AMP, consequently suppressing the effect of agents acting through cyclic AMP¹⁰. The effect of 10⁻⁶ M PGE₂ was studied in the presence of 4×10^{-3} M imidazole. The culturing system was identical with that described above. Neutralised imidazole was added to the cultures at the start of incubation. Table 1 shows that imidazole had no effect on PGE₂-induced increase in the number of CFU in S phase.

TABLE 1 Effect of imidazole on PGE₂-induced increase in number of CFU in S phase

	CFU/6 × 10 ⁴ cells		
	Incubated without ³ H-thymidine*	Incubated with ³ H-thymidine*	Killing effect %
Control	11.25 ± 2.66 12.87 ± 2.16	10.77 ± 3.05 12.14 ± 2.87	4.0 6.0
PGE ₂ (10 ⁻⁶ M)	11.12 ± 1.56 12.67 ± 2.96 12.50 ± 2.30	7.25 ± 2.95 6.60 ± 1.36 9.74 ± 1.76	35.0† 48.0† 28.0†
PGE ₂ (10 ⁻⁶ M) + Imidazole (4 × 10 ⁻³ M)	10.50 ± 1.96 11.10 ± 1.66	7.22 ± 1.48 5.60 ± 1.02	31.0† 49.0†
	11.90 ± 2.10	8.66 ± 1.87	27.0†

* Mean ± s.d.

† *P* < 0.01

‡ *P* < 0.05

The experiments reported suggest PGE₂ to be a potent non-toxic stimulator of the proliferation of haemopoietic stem cells *in vitro*. The effective PGE₂ concentrations *in vitro* (10⁻¹³ M to 10⁻⁴ M, that is, 0.035 pg ml⁻¹ to 35 × 10⁶ pg per ml⁻¹) fall in the range of PGE level proved to be present in human serum (316 ± 36 pg ml⁻¹)¹¹. If administered in higher concentrations (minimum 10⁻⁵ µg per g body weight), though still within the physiological range, this triggering effect on CFU can also be demonstrated *in vivo* (unpublished data).

The cell cycle action of PGE₂ on haemopoietic stem cells is through some mechanism different from cyclic AMP since it cannot be prevented by imidazole. This effect of PGE₂ resembles the stimulatory effect exerted by testosterone on CFU proliferation which also does not act through the cyclic AMP system¹².

Summarising the previously published data^{3,8,13} and the present experiments, the correlation among PG, cyclic-AMP-system and cellular proliferation seems to differ according to cell types. (1) Prostaglandin enhances the intracellular cyclic AMP level and mitotic activity in thymocytes³. (2) It may induce cyclic AMP accumulation in lymph node cells too, without causing their blast-transformation or mitosis.⁸ (3) PGE₂ triggered resting (G₀) haemopoietic stem cells into the cell cycle even if increased cyclic AMP destruction had been induced. (4) Finally, PGE may restore controlled growth of transformed cells¹³ through the cyclic AMP system.

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I. FEHÉR
JULIA GIDÁLI

'Frédéric Joliot-Curie'
National Research Institute
for Radiobiology and Radiohygiene
Budapest 22

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Immunosuppressive Effect of an Anti-interferon Serum

INTERFERON has been reported to play an important role in immune responses¹⁻⁶. Using interferon^{3,5,6} acceleration of mouse skin allograft rejection has been demonstrated in addition to an increase in the cytotoxic action of lymphocytes on appropriate target cells *in vitro*. It has also been shown that prolongation of skin allografts can be achieved by the use of anti-interferon serum³. To further test the possibility of involvement of interferon in immune responses an attempt is here described to block the interferon effect by anti-interferon serum.

Anti-interferon serum was obtained by immunisation of a goat with the interferon induced in L cell culture by the Newcastle disease virus (NDV). The titre of the crude preparation was 800 to 1,200 units ml⁻¹. Before immunisation, blood was collected from the goat and the control serum was prepared. The animal received forty-nine weekly subcutaneous injections of the crude interferon. Later interferon preparations were purified and concentrated before use.

Initially purification and concentration were as follows: liquid containing interferon was centrifuged at 18,000 r.p.m. for 20 min and then concentrated ten-fold using polyethylglycol (molecular weight 40,000). Subsequently the interferon was purified by passage through Millipore filters of 1.2 µm or 0.45 µm diameter and concentrated by UM-2 (Amicon) filtration. The titre of the interferon sample obtained in this way was 6,000 to 8,000 units ml⁻¹. Goats were thus given 60,000 to 80,000 units of interferon in a single subcutaneous injection. After twenty-two injections the goat serum revealed antibodies to the interferon.

The serum was stored at -30° C. Before use it was adsorbed with an L-cell suspension (3 × 10⁶ cells ml⁻¹ serum) at 37° C for 1 h and then at 4° C for 24 h and similarly with lymphocytes of C57Bl/6 mouse lymph nodes (3 × 10⁶ cells ml⁻¹ serum).

The sera were then tested in a microcytotoxicity test and showed no toxic activity on L cells or lymphocytes.

For titration of anti-interferon serum, a series of two-fold dilutions of control and immune sera from 1 : 2 to 1 : 256 in TC 199 supplemented with 2% inactivated bovine serum was made. To each serum dilution, 8 units of the interferon were added in an equal volume and the mixture was incubated at 37° C for 1 h and at 4° C for 24 h. Then the contents of each tube were placed on the L cell monolayer (the cells had been seeded the day before) and incubated at 37° C for 18 to 20 h, whereupon 100 TCD₅₀ of the test-virus (VSV) was added. Results were evaluated 24 h after the challenge with the virus. The titre of the anti-interferon serum was expressed as the maximum dilution which still neutralised the protective effect of the interferon. The anti-interferon serum employed showed a titre of 1 : 64. The control serum did not reveal any protective activity.

Four groups of female C57Bl/6 mice were used. Mice of group I received a single injection of 7 × 10⁶ L cells subcutaneously in each of eleven sites and, simultaneously, 0.3 ml of anti-interferon serum intraperitoneally. This volume of anti-interferon serum was administered daily for 5 d in all. Group II was immunised with L cells and the control serum was given using the same time course of injections. Group III was similarly immunised with L cells only and group IV remained intact.

Table 1 Cytotoxic Action of C57Bl/6 Mice Lymph Node Lymphocytes on L Target Cells.

L cells + anti-interferon serum (I)	Immunisation		
	L cells + normal serum (II)	L cells (III)	Intact (IV)
97,765 ± 6,490 *	59,994 ± 3,850	38,885 ± 4,730	143,319 ± 9,900

* Mean number of viable L cells ml⁻¹.

At 7 to 10 d after the onset of immunisation, lymph nodes were taken from all the mice and the cytotoxic activity of the lymphocytes was investigated. Suspensions of lymphocytes were added to the 24 h monolayer of L target cells in the ratio proportion of 50 : 1. After 48 h incubation, cells were collected by treatment with 0.25% trypsin and their viability determined by staining with eosin-trypan blue. Statistical significances were estimated by the Student's *t* test.

As seen from Table 1, in mice given anti-interferon serum in addition to L cell immunisation (group I) lymphocyte cytotoxicity to L target cells was suppressed as compared with that of lymphocytes from mice given normal goat serum (group II) (*P* < 0.01).

The sera of experimental and control groups were tested in parallel in a microcytotoxicity test with undiluted guinea pig complement⁷. Terasaki plates (well size 10 µl) were used. The serum (1 µl) was incubated with L cells (1,000 to 1,500 cells per well). After 30 min incubation at 37°, 5 µl of the complement (undiluted guinea pig serum) were added and the mixture was incubated for an additional 1.5 h. The supernatant was then shaken from the wells and supravital stain was added. After 3 min exposure, the stain was removed and cells were counted. The reaction was evaluated as follows: -, 0 to 15% of dead cells; ±, 16 to 20% of dead cells; +, 21 to 30% of dead cells; 2+, 31 to 50% of dead cells; 3+, 51 to 80% of dead cells; 4+, more than 81% of dead cells.

As seen in Fig. 1, the sera of mice immunised with L cells, and those of the animals immunised with L cells and given normal goat serum, showed a cytotoxic activity to L cells in a titre of 1 : 40. In contrast, the sera of mice immunised with L cells plus anti-interferon serum did not reveal any cytotoxic activity.

It has therefore been demonstrated that the administration of anti-interferon serum can induce an immunosuppressive effect

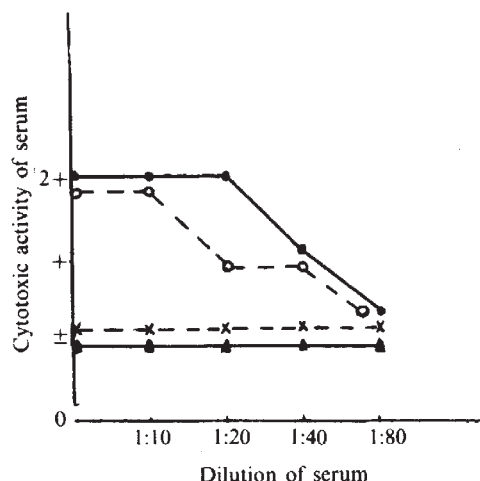


Fig. 1 Cytotoxic action of C57Bl/6 mouse serum on L target cells. ●, Mouse serum after L cell immunisation; ○, mouse serum after L cell immunisation and administration of control serum; ×, mouse serum after L cell immunisation and administration of anti-interferon serum; ▲, control untreated mouse serum.

which is revealed both at the level of cellular and humoral immunity. A certain decrease in the cytotoxicity of lymphocytes of mice receiving normal goat serum may be accounted for by the concurrent action of the serum with the immunising agent (L cells). On these grounds it seems possible that interferon is a prerequisite for the full expression of the response to an antigenic stimulus. Also, antigens on this basis are interferogens⁴. We therefore believe that interferon may play a part in the recognition of a specific antigen by an immune lymphocyte and may be a necessary factor involved in the interaction between the antigen and a specific effector cell or antibody.

The exact manner in which interferon is involved in the immune process is not indicated in the present experiments, but it is intriguing to contemplate the possibility that autoimmune (allergic) diseases are connected with the abundant interferon production and anti-interferon serum might be used for the treatment of autoimmune disease. It is also our belief that the immunosuppression which is sometimes observed in cancer patients and which has, by some authorities, been thought responsible for the malignant conditions, is associated with inhibition of interferon synthesis.

S. V. SKURKOVICH
E. G. KLINOVA
E. I. EREMINKA
N. V. LEVINA

Central Institute of Haematology
and Blood Transfusion,
Moscow

Received June 4; final revision September 19, 1973.

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In vitro assembly of 30S ribosomal particles from precursor 16S RNA of *Escherichia coli*

THE concept of self-assembly has been shown to apply to the assembly of the *Escherichia coli* 30S ribosome *in vitro* by Nomura and his co-workers^{1,2}. The reconstitution of functional 30S ribosomes from purified mature 16S RNA (m16 RNA in the nomenclature of Hecht and Woese³) and total 30S proteins has been studied in detail. The macromolecular information necessary for this *in vitro* assembly clearly resides in the component parts with no requirements for enzymes or cellular structures (such as membranes) which are not ultimately part of the 30S ribosome. The reconstitution system developed by Traub and Nomura¹ has been useful in a variety of studies which have greatly aided our understanding of ribosome assembly, structure, and function. The biosynthesis of ribosomes, however, is known to differ in several ways from this *in vitro* reconstitution. The *in vivo*

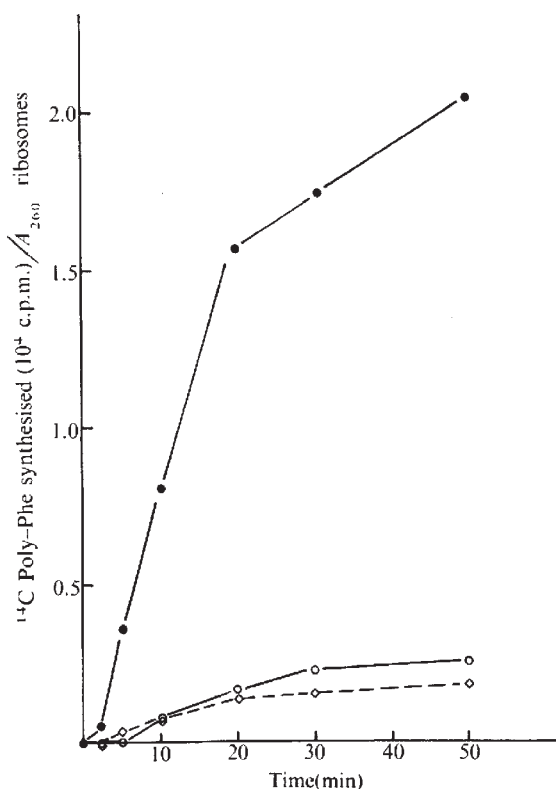


Fig. 1 Kinetics of incorporation of ¹⁴C-phenylalanine into hot TCA-precipitable peptide. A 1.25 ml reaction mixture, prepared as described⁶, was initiated at 37° C with poly (U). The 30S ribosomes derived by reconstitution were mixed with mature 50S ribosomes. Reconstituted ribosomes were prepared as described⁶ using m16 RNA and p16 RNA. The RNA's were prepared from a culture of *E. coli* D10 (Met-RC relaxed) which had been deprived of methionine for 90 min. The m16 RNA was labelled by incorporating ³H-uracil during the exponential growth phase. The p16 RNA was labeled with ³²P_o during the period of methionine deprivation. RNA was extracted from the cells with SDS and phenol and applied to a 5-20% sucrose gradient. The 16S region was recovered and the RNA was precipitated with ethanol. The RNA was then dissolved in Tris-HCl buffer and applied to 3.2% polyacrylamide gels with a diameter of 20 mm. The conditions of electrophoresis were described by Bishop *et al.*¹⁴ The peaks of m16 and p16 RNA were recovered after the gels had been sliced and eluted into buffer. Appropriate fractions were pooled to give a p16 RNA preparation that had 9% m16 RNA present. There was no detectable p16 RNA in the m16 RNA preparation. The poly (U) assays contained 0.45 A₂₆₀ units of 30S ribosomes. ●—●, 30S particles containing m16 RNA; ○—○, 30S particles containing p16 RNA; ◇—◇, 9% of the activity of m16-containing particles.

process is composed of a series of cooperative and sequential interactions between the primary ribosomal RNA transcripts and of ribosomal proteins, as well as the modification of both RNA and protein^{4,5}.

We have attempted to assess the role that precursor 16S RNA (p16) might play in the *in vivo* assembly of ribosomes by carrying out reconstitution experiments with this form of

RNA and the proteins derived from mature ribosomes. We chose the unmethylated precursor which accumulates during methionine deprivation of a relaxed auxotroph of *Escherichia coli*. Using purified p16 RNA or a differentially labelled mixture of m16 and p16 RNA, we have shown that p16 RNA assembles to a homogeneous 30S particle containing all the ribosomal proteins except S3 (and possibly S6) and that such particles are inactive in a poly (U) incorporation assay (unpublished).

In this report, we have examined the possibility that P16 RNA might be preferred to mature 16S RNA (m16) in the reconstitution process, and that the use of p16 RNA might lower the unusually high activation energy found for the assembly 30S particles with m16 RNA². Total 30S ribosomal proteins and p16 RNA, under the conditions described by Traub *et al.*⁶ form a particle which co-sediments with native 30S ribosomes (see Fig. 2d). There is no poly (U) directed biosynthesis of polyphenylalanine attributable to the particles made with p16 (Fig. 1).

The p16 RNA was competed with m16 RNA in the reconstitution system to determine which was the preferred substrate for the interaction with proteins. When the amounts of total 30S proteins were limiting (Fig. 2, a-c) the m16 RNA was preferentially assembled into particles when both p16 and m16 RNA were present. Only at higher concentration of protein did the p16 RNA become incorporated into a homogeneous particle. Thus, in this system, m16 RNA is clearly more efficient than p16 RNA in interacting with proteins and forming an RNP particle.

We examined the possibility that p16 RNA might lower the activation energy for the reconstitution process by carrying out the reaction at various temperatures. Because particles with p16 RNA have no protein synthesizing capacity, we had to devise an alternative method for measuring the end point of the assembly. Both p16 and m16 RNA are highly sensitive to pancreatic RNase, but when assembled into RNP particles, become relatively resistant. Therefore, the temperature-dependent acquisition of RNase resistance was used as a measure of the overall reconstitution process for both types of RNA. As Fig. 3 shows, there is no discernable difference between the RNAs in the temperature dependence of their reconstitution into particles. Therefore, p16 RNA does not have a lower activation energy for assembly as measured by the thermal energy required to give complete 30S particles when the reconstitution is carried out with excess proteins.

There are at least two major differences between the p16 and m16 RNAs. The precursor rRNA contains excess oligonucleotides at both its 3' and 5' ends and also lacks all of the fifteen or so methyl groups found in m16 RNA⁷⁻¹⁰. The relative contribution of the methylated sequences of m16 RNA

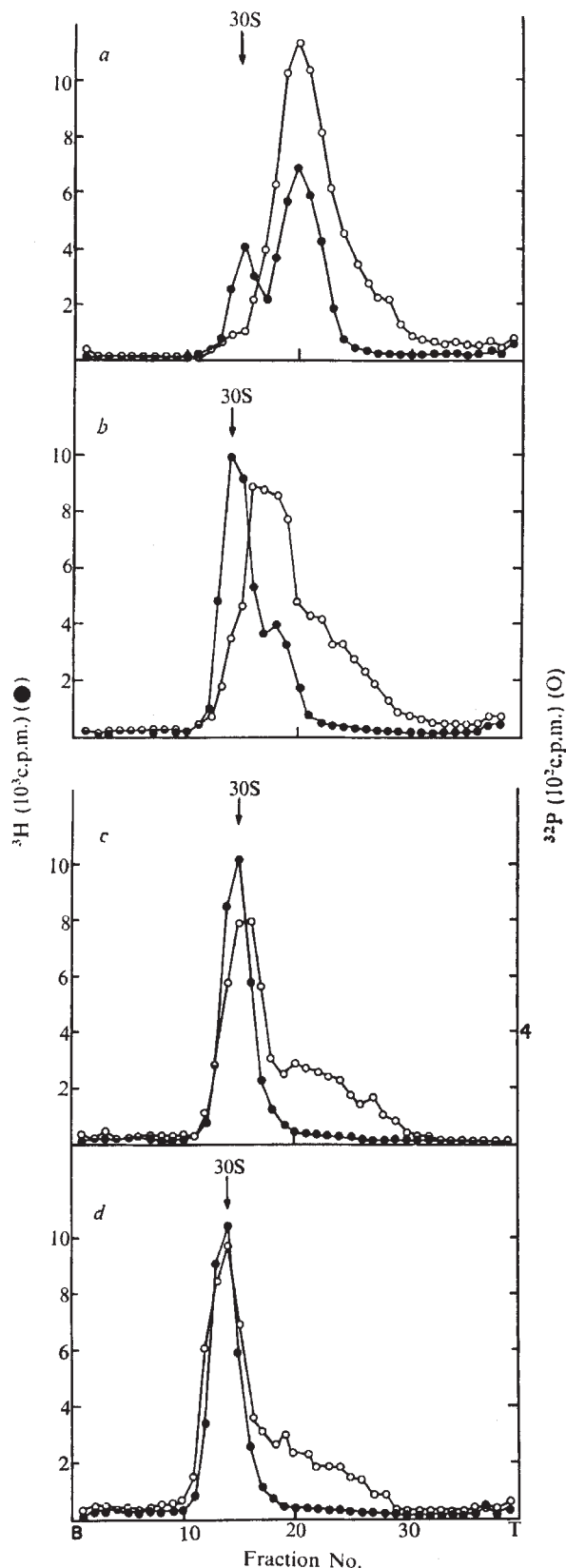


Fig. 2 Competition between m16 and p16 RNA for limiting 30S proteins. Reconstitution reactions with 0.2 A_{260} units of a mixture of ^3H -uracil-labelled m16 RNA (●—●) and ^{32}P labelled p16 RNA (○—○) were incubated with increasing amounts of total 30S proteins. The reconstitution reactions were done, as previously described⁶ at 40° C for 20 min. Each reaction was cooled to 0° C and layered directly on 5 ml 10–30% tris- Mg^{2+} sucrose gradients (10 mM Tris-HCl, pH 7.8 and 0.3 mM MgCl_2). The gradients were run 3 h at 49,000 r.p.m. in a SW 50.1 rotor. The gradients were pumped out with an Isco density gradient fractionator and the radioactivity in each fraction of the gradient was determined by liquid scintillation spectroscopy. a, 23 μg 30S protein per A_{260} unit of mixed 16S RNA; b, 35 μg protein; c, 46 μg protein; and d, 69 μg protein. In this experiment a portion of the ^{32}P label is slow sedimenting and does not reconstitute to a 30S particle. Other experiments with this variable RNA fraction have shown that it contains internal nucleolytic cleavages (by melting experiments) and probably represents mRNA or degraded p16 RNA.

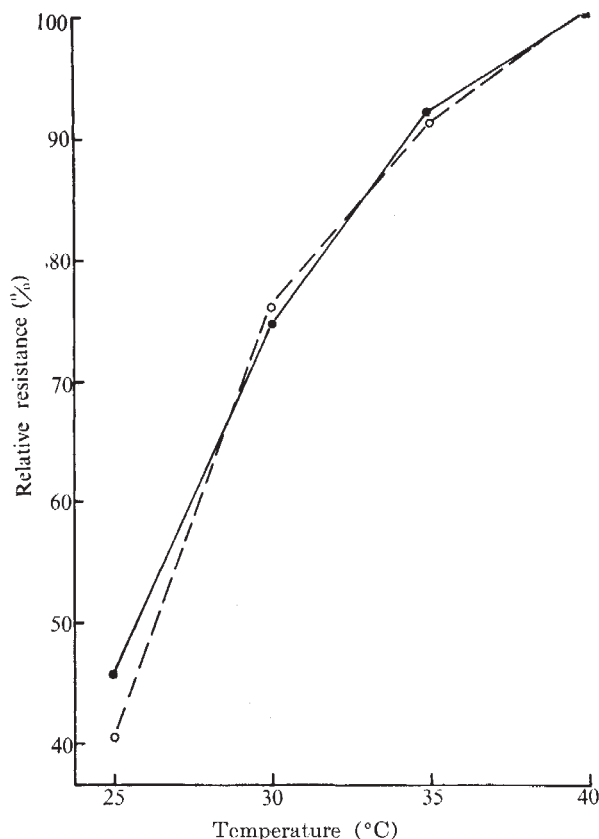


Fig. 3 Temperature dependence of reconstitution with m16 RNA and p16 RNA. Reconstitution was measured by the relative resistance to digestion by pancreatic RNase of reconstituted particles. Reactions⁶ were done with 0.72 A_{260} units of a mixture of m16 (^3H -labelled) and p16 (^{32}P -labelled) RNA, and 44 μg total 30S proteins (61 μg per A_{260} unit). The reactions were incubated at 25° C, 30° C, 35° C, and 40° C, respectively, for 20 min in a volume of 0.5 ml. A control reaction with RNA alone was incubated at 40° C for 20 min. After the incubation, duplicate 0.2 ml aliquots of each reaction were diluted forty-five-fold in 8.8 ml of buffer composed of 5 mM Tris-HCl, pH 7.8, 5 mM MgCl_2 and 50mM NaCl at 25° C. After withdrawing duplicate 0 time aliquots of 2 ml from each reaction, 1 μg of pancreatic RNase was added and the incubation continued for 50 min at 25° C. After 50 min of incubation in the presence of RNase, duplicate aliquots of 2 ml were withdrawn. The zero time and 50 min aliquots were precipitated with 2 ml of 10% TCA containing 1 mg ml^{-1} bovine serum albumin at 0° C. After 20 min at 0° C, the precipitates were collected on glass fibre filters and washed with 40 ml of 5% TCA. The precipitable radioactivity was determined for each aliquot and the mean RNase resistance for each reconstitution reaction temperature (mean c.p.m. of four aliquots incubated with RNase for 50 min) was determined relative to the zero time measurement. The change in resistance for each reaction condition is the resistance measured minus the resistance of RNA reacted in the absence of proteins with the resistance for the reaction at 50° C taken as 100% (actual change in resistance was 48.2% for m16 and 24.5% for p16). The other reaction temperatures were plotted relative to this maximum. ●—●, m16; ○—○, p16.

to its preferential protein binding during assembly cannot be determined as yet. The preferential assembly of m16 RNA to a 30S ribosome could however be due to the cooperative nature of assembly^{11,12}. The precursor RNA could be present as a population of random conformations (never having been assembled to a 30S ribosome as has m16 RNA) of which only a small proportion are in a conformation suitable for the binding of early proteins, and subsequently the late binding proteins which are required for structural assembly of a 30S ribosome. It has been shown that ribosomal proteins do

affect the conformation of ribosomal RNA¹³. If proteins are present in excess, then there is a rapid shift away from the equilibrium condition among the random conformations towards the specific conformation which is required for the initiation of assembly (rapid because the assembly rates of m16 RNA are apparently similar, Fig. 3). Because a precursor form of RNA is found in immature RNP particles, themselves precursors to ribosomes, *in vivo* assembly is likely to couple protein binding, RNA methylations and oligonucleotide cleavage of p16 in a cooperative and sequential manner. A study of such interactions may be possible using p16 RNAs with varying degrees of methylation, and by carrying out the simultaneous nuclease trimming of the RNA.

This work was supported by grants from the National Institutes of Health and from the National Science Foundation. One of us (J.W.W.) was supported by a stipend from a training grant awarded to the Department of Microbiology, University of Illinois, Urbana. Portions of this work come from a thesis submitted to the University of Illinois, in partial fulfillment for the requirements for the Ph.D. degree.

JOHN W. WIREMAN

PAUL S. SYPHERD

Department of Medical Microbiology,
College of Medicine,
University of California, Irvine,
Irvine, California 92664

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Dual Role of Gene D5 in the Development of Bacteriophage T5

THREE distinct classes of phage-specific proteins—"pre-early", "early" and "late"—are synthesised in a temporal sequence after infection of *Escherichia coli* with bacteriophage T5¹ (Fig. 2a). The synthesis of pre-early proteins begins within the first minute after infection and is shut off 8–10 min later^{1,2}. The beginning of synthesis of early proteins is delayed until about 6 min after infection. This delay is mechanical, and is a result of the transfer of phage DNA to the host cell in two steps^{1,3}. The rate of synthesis of early proteins decreases considerably after 20 to 25 min of infection, but synthesis does continue through the late period at diminishing rates. We have observed that the rate of synthesis of most early proteins is diminished normally even when the turn-on of synthesis of late proteins is defective⁴. This observation suggested to us the presence of a negative control mechanism for the 'shut-off' of synthesis of early proteins, rather than a passive shut-off, such as a competition between early and late transcripts for the translational

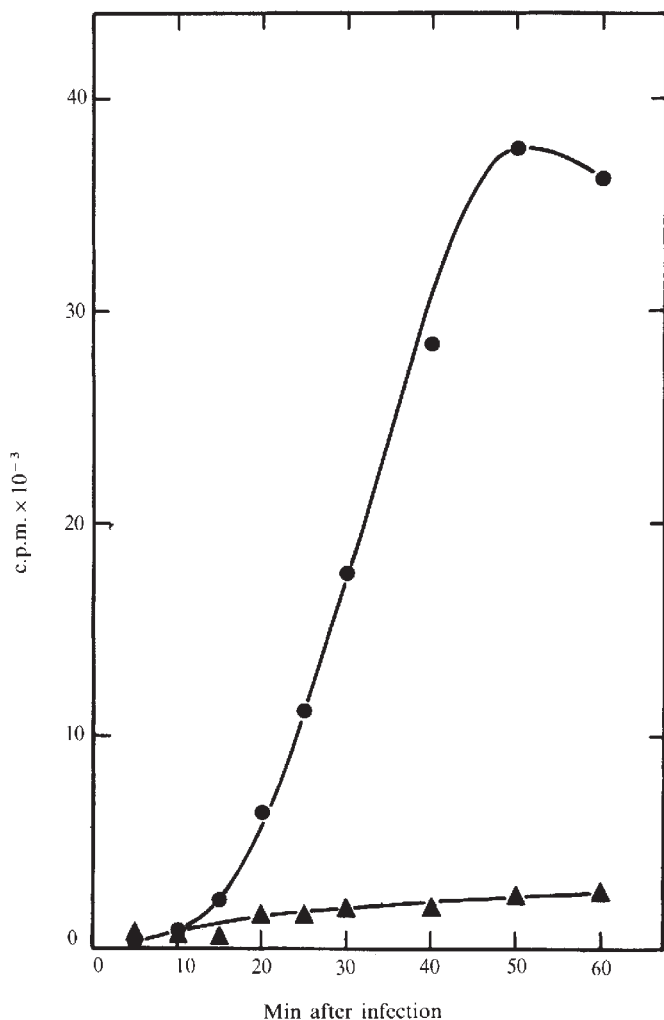


Fig. 1 Cumulative incorporation of ^3H -thymidine into DNA after infection of *E. coli* F (su^-) cells with T5^+ (●) and with T5(D5)amH12a (▲). Bacteria were grown, concentrated and infected as described in ref. 5. The phage-bacterium complexes were diluted in a malate-glucose-salts medium containing 10^{-3} M CaCl_2 , 250 μg of deoxyadenosine ml^{-1} , 5 μg of uracil ml^{-1} , and 2 μg of ^3H -thymidine ml^{-1} (specific activity, 1.75 μCi μg^{-1}). Aeration was begun immediately after dilution ('zero time') and 1.0-ml samples were taken at various times and added to 1.0 ml of ice-cold 15% trichloroacetic acid. 0.03 ml of 0.5% serum albumin and 0.05 ml of 0.25% salmon sperm DNA were added as coprecipitants. After at least 30 min at 0°C , the acid-insoluble material was collected on glass fibre filters (Reeve Angel, Grade 984 H), and washed successively with 10 ml of 5% trichloroacetic acid, 10 ml of 0.5% trichloroacetic acid and 10 ml of acetone. The filter pads were dried and counted in a liquid scintillation counter using a toluene-based scintillation fluid.

system of the infected cell. We have identified a gene the product of which is required for the shut-off of synthesis of phage specific early proteins, but which is also required for the replication of phage DNA.

Figure 1 shows that phage DNA is not synthesised in non-permissive (su^-) cells infected with an amber mutant defective in gene D5, whereas phage DNA synthesis begins at about 10 min after infection and continues for 40 min in cells infected with wild-type phage. Figures 2 and 3 show that the synthesis of all early proteins the synthesis of which are shut off by about 25 min after infection with wild-type phage, is not shut off after infection with a mutant defective in gene D5, with the exception of polypeptide E13. The rate of synthesis of E13 is nevertheless still appreciable 60 min after infection, although it is less than at 13 min after infection. The synthesis of early

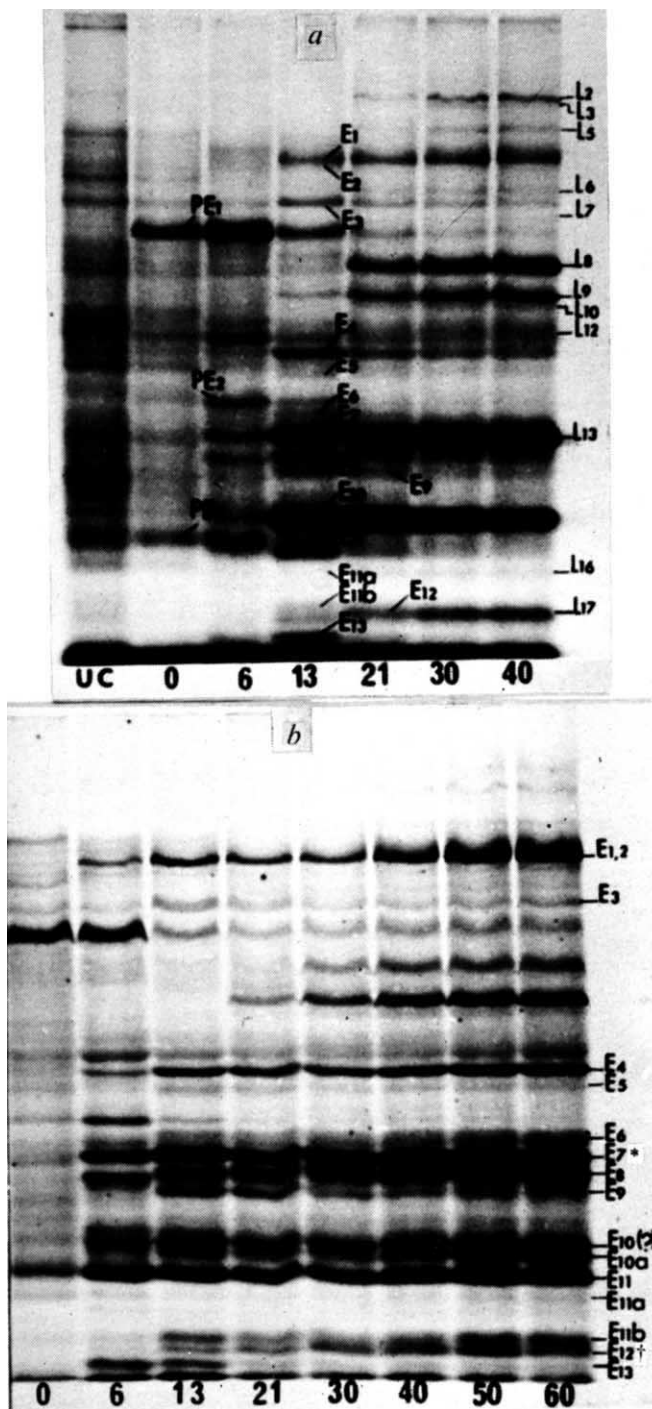


Fig. 2 Autoradiograms of electrophoretically separated ^{14}C labelled polypeptides from *E. coli* F (su^-) infected with T5. The electrophoresis was performed in 10% polyacrylamide gels containing sodium dodecyl sulphate (SDS). *a*, T5^+ ; *b*, T5(D5)amH12a . *E. coli* F cells were grown and infected as described in ref. 5. Samples of phage-infected cultures were pulse-labelled essentially as described in ref. 1 with ^{14}C -reconstituted protein hydrolysate (0.2 μCi ml^{-1}). Electrophoresis and autoradiography were carried out as described in refs 4 and 5. The numbers below the autoradiograms indicate the times when 4-min pulses were begun. The designation UC, PE, E, and L represent uninfected cells, pre-early, early, and late polypeptides, respectively. For details concerning the pre-early and late polypeptides see refs 6 and 5, respectively. * Polypeptides E7 and L13 run together in 10% gels, but can be resolved in 15% gels. Analysis of pulse-labelled polypeptides in 15% gels revealed that the synthesis of polypeptide E7 is shut off by about 25 min after infection with T5^+ , whereas it is not shut off by 60 min after infection with T5(D5)amH12a . † In cells infected with T5(D5)amH12a , L17 is absent due to a defect in the cleavage of a head protein. Polypeptide E10 (?) in (*b*) is probably a polypeptide that has an electrophoretic mobility very similar to polypeptide E10.

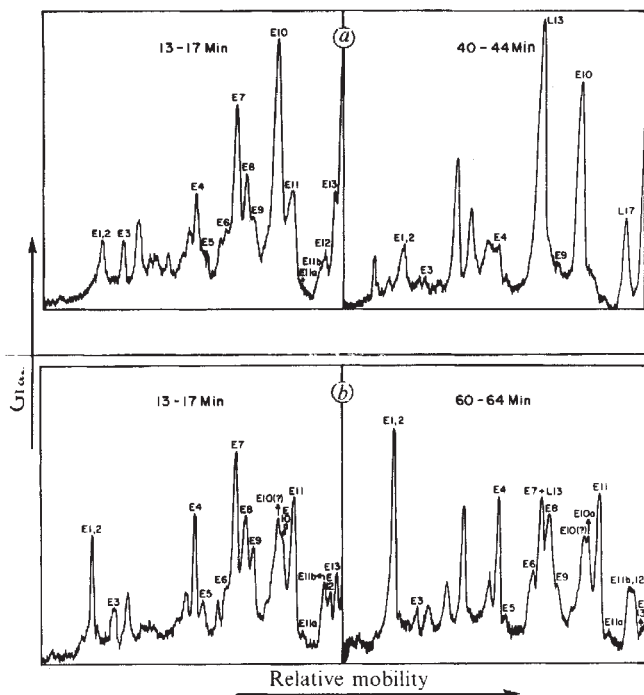


Fig. 3 Microdensitometer scans of the autoradiograms of electrophoretically separated ^{14}C -proteins from su^- cells infected with T5 $^+$ (a) and T5(D5)*am*H12a (b) (see Fig. 2). The designation E identifies early polypeptides. Scans were performed with a Joyce-Loebl microdensitometer.

proteins E1, E2 and E10 is not shut off at late times even in infections with wild type phage (Fig. 2a). Apparently, the synthesis of most early proteins is shut off by a mechanism involving the product of gene D5, but the synthesis of at least one early protein (E13) is shut off partly by another mechanism. Other DO (no phage DNA synthesis) mutants of T5 (genes D7, D8, and D9^{7,8}) show normal shut-off of the synthesis of most early proteins, although one or two proteins do show slightly extended synthesis (our unpublished data).

The product of gene D5 is most likely polypeptide E10. It is synthesised in such large amounts that it masks two or three other polypeptides that have electrophoretic mobilities very close to it (Fig. 2a). When E10 is not synthesised, these other two or three polypeptides can be seen (Fig. 2b). Our preliminary results indicate that polypeptide E10 is a DNA-binding protein. Its role in the replication of phage DNA may be similar to that of other phage-induced DNA-binding proteins⁹⁻¹². Its effect on the synthesis of early proteins may be at the level of transcription where it could act as a repressor for the synthesis of early mRNA. We are investigating the effect of the product of gene D5 on the transcription of early T5 genes *in vivo* and *in vitro*.

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G. CHINNADURAI
D. J. MCCORQUODALE

Institute for Molecular Biology,
University of Texas at Dallas,
Dallas, Texas 75230

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On the Evidence for Partial Gene Duplication from Amino Acid Sequence of Bovine Glutamate Dehydrogenase

BOVINE glutamate dehydrogenase consists of a single polypeptide chain of 500 residues¹. Engel² has proposed that the regulatory site of the enzyme was produced by duplication of the amino acid sequence of the active centre, because when sections 114-163 and 269-318 are aligned there are twelve positions where identical amino acids occur in the two sections. Engel calculated that the probability of observing twelve identical positions in two random sequences of length fifty residues was 4.3×10^{-6} . He argued that as there are 352 possible sequences of fifty residues with which section 114-163 may be aligned without overlapping itself the overall probability is $352 \times 4.3 \times 10^{-6}$ ($= 1.5 \times 10^{-3}$). On this basis the observation quoted above is highly significant. We will show here that this probability is not significant.

Using a computer twenty random sequences of length 500 residues were constructed. Ten of these random sequences were drawn from an infinitely large amino acid pool with the same composition as the enzyme. Another ten sequences were constructed by 'random rearrangement' of the amino acid sequence of glutamate dehydrogenase. The compositions of the latter group of random sequences were, therefore, identical with that of the enzyme whereas in the former group the random sequences did not possess precisely the same composition as the enzyme. Taking the actual amino acid sequence of bovine glutamate dehydrogenase and the twenty random sequences we have aligned all possible pairs of sequences of length fifty residues. For each 500 residue sequence there were 101,475 alignments. The number of identical positions was counted for each alignment (Table 1). For the two groups of random sequences Table 1 shows means and standard deviations. In all cases except one the observed frequencies for glutamate dehydrogenase lie within two standard deviations of the means of the frequencies for the random sequences.

Therefore, by this test, Engel's finding of twelve identical positions in two runs of fifty residues is not significant and we conclude that there is no evidence for duplication in the amino acid sequence of glutamate dehydrogenase.

It remains possible that different ways of comparing sections of the sequences, for example by allowing the use of a small number of gaps to increase the number of identical positions or by using the theoretical minimum number of nucleotide base changes might yield a different conclusion. The use of these methods, however, also involves uncertainties and difficulties in interpretation as has been pointed out by Haber and Koshland³ and by Dickerson⁴.

Several authors have pointed out that even random sequences

contain unique, but unpredictable, regularities⁵. Are sections 114–163 and 269–318 therefore the parts of the sequence which Engel's theory would predict to be homologous? There is no

even fifty may be an overestimate of the necessary number of sequences containing lysine-126 to be compared.

(2) Even if one concedes a probability as high as 7.5% for random occurrence of the observed degree of matching for a sequence including lysine-126, this takes no account of the additional evidence from the comparison with GPDH⁵. Here there can be no question of screening the whole molecule: the alignment is already fixed on the basis of the observed homology of sequences surrounding the lysine residues in GDH and GPDH that react with PLP^{6,7}. The two GDH sequences are, of course, aligned on the basis of the internal homology. The probability of the observed matching of nine positions out of sixty-four in GDH 2 and GPDH may be calculated as follows.

The match^{6,7} between GDH 1 and GPDH is assumed to reflect a genuine evolutionary relationship (six identities out of sixty-four). The match⁵ between GDH 1 and GDH 2 (thirteen identities out of sixty-four) is taken, for the purposes of calculation, to be a fortuitous occurrence judiciously selected. The probability, higher than for twelve out of fifty, is 0.21, based on 64×310 comparisons. (For such large numbers of comparisons the approximation of multiplying the probability calculated for a single comparison by the number of comparisons breaks down and the second term in the binomial expansion must be included.)

Thirteen random matches with the sixty-four residues of GDH 1 could coincide with 0, 1, 2, 3, 4, 5 or 6 of the matching positions in the GDH 1/GPDH comparison. If no positions match in all three sequences, this defines $13 + 6 = 19$ positions that differ in GDH 2 and GPDH. Thus nine matches would have to be found among the remaining forty-five pairs of residues. Similarly, one congruence in all three sequences defines eighteen positions that differ in GDH 2 and GPDH, so that eight identities must be found in forty-five comparisons, and so on. The probability of finding n positions identical in all three sequences is given by

$$P_1 = \frac{{}^{58}C_{(13-n)} \times {}^6C_n}{{}^{64}C_{13}}$$

The probability of finding $9-n$ further positions matching in GDH 2 and GPDH is given by

$$P_2 = \frac{{}^{(45+n)}C_{(9-n)} \times {}^{19}C_{(36+2n)}}{{}^{20}C_{(45+n)}}$$

The overall probability of the total of nine matches that is in fact found is

$$\sum_{n=0}^6 P_1 P_2 = 4.2 \times 10^{-3}$$

The cumulative probability, therefore, of the two observed matches with GDH 2 is $4.2 \times 10^{-3} \times 2.1 \times 10^{-1} = 8.8 \times 10^{-4}$.

To say the least this signifies a noteworthy coincidence. The availability of more sequence data should soon provide further evidence for or against the hypothesis of partial gene duplication in GDH.

P. C. ENGEL

Department of Biochemistry,
University of Sheffield, S10 2TN

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Table 1 Frequencies of Different Numbers of Identical Positions

No. of identical matches	Observed frequencies		
	Bovine GDH	Rearranged Random sequences	Drawn from pool
0	5,155	5,270 ± 321	4,721 ± 406
1	15,828	15,987 ± 360	14,881 ± 952
2	23,604	23,983 ± 359	23,077 ± 277
3	23,307	23,284 ± 568	23,516 ± 482
4	17,026	16,755 ± 329	17,433 ± 727
5	9,878	9,402 ± 235 *	10,149 ± 836
6	4,504	4,357 ± 231	4,829 ± 553
7	1,508	1,712 ± 146	1,918 ± 208
8	464	546 ± 69	663 ± 102
9	164	135 ± 25	123 ± 59
10	34	32 ± 23	56 ± 29
11	2	5.7 ± 7.8	11.3 ± 8.2
12	1	1.4 ± 2.9	2.1 ± 3.6
13	—	0.1 ± 0.3	1.6 ± 4.8
14	—	—	0.1 ± 0.3

* Observed frequency more than two standard deviations from mean of frequencies for random sequences.

current evidence that section 269–318 constitutes the regulatory site and for the active centre the only definite fact is that lysine 126 is essential for enzyme activity. Since there are fifty sequences of length fifty residues which include residue 126, Engel's calculated probability for the random occurrence of twelve identical positions should be $50 \times 1.5 \times 10^{-3}$ ($= 7.5 \times 10^{-2}$) and on this basis the observation quoted is not significant. It is not however to be seriously supposed that either the active centre or the regulatory site would correspond to fifty contiguous residues.

Although no valid evidence has so far been produced in its favour it is possible that Engel's proposal as to the origin of the regulatory site of enzymes is correct and the evidence from X-ray studies may prove important in this problem.

J. WILLIAMS
A. G. WILKINS

Molecular Enzymology Laboratory,
Department of Biochemistry,
University of Bristol,
Bristol BS8 1TD

Received April 18; revised October 29, 1973.

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Dr ENGEL replies:

Williams and Wilkins have done the evidence less than justice in two respects: (1) Our disagreement hinges upon the choice of a basis for statistical assessment of the observed sequence match. Absolute validity can neither be attained nor defined; one can only attempt to select reasonable criteria. My original probability calculation may have been too arbitrarily exclusive but, equally, Williams's and Wilkins's computer-search is too arbitrarily all-inclusive. To ignore relevant information must bias the calculation against discovery of the truth. The evidence for the importance of lysine-126 comes not only from studies of kinetics and binding (see ref. 1), but also from sequence comparisons which show that lysine-126 is in a conserved region^{2–4}. This being so,

Conformational Analysis of N⁶-Methyladenylyl-Uridine

THE availability of a large Fourier transform (FT) NMR system in the laboratory of one of us (G. v. B.) allowed us to obtain a series of proton magnetic resonance spectra of nucleosides and dinucleoside monophosphates superior to any hitherto reported. We shall show that the use of the FT-technique at 270 MHz can increase our understanding of the structural features and of the dynamics of the stacking (helix-forming) processes because the chemical shifts as well as the ¹H-¹H and the ¹H-³¹P coupling constants hidden in the high-field (ribose) part of the spectrum are now in principle amenable to analysis. These parameters provide the key to unlock the secrets of the three-dimensional time-average structure of dinucleosides in solution.

We report here the first complete assignment of the ribose protons of a dinucleoside monophosphate, N⁶-methyladenylyl-uridine ammonium salt (m⁶ApU, or N⁶-MeApU, Fig. 1) and we propose a model for the various conformational equilibria based on recent studies on the pseudorotation properties of the ribose ring^{1,2}.

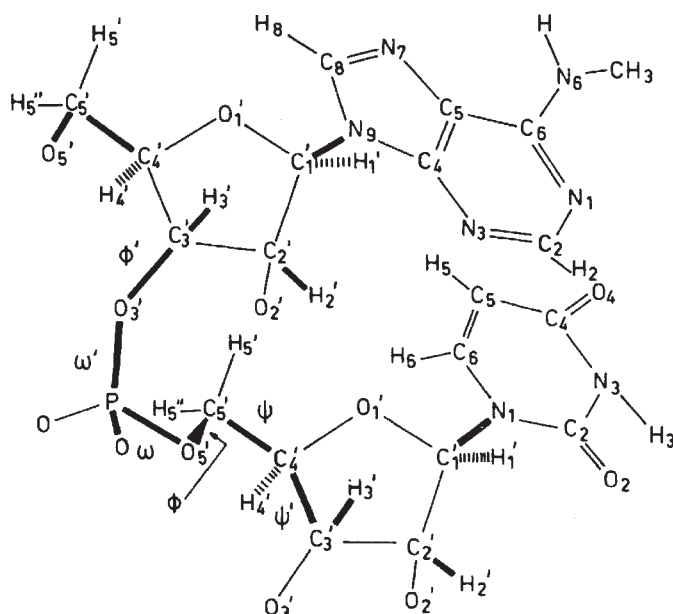


Fig. 1 Structure of N⁶-methyladenylyl-uridine, showing the stereochemical relationships between the coupled nuclei as well as the principal backbone torsion angles¹¹.

This work forms part of our programme of research into the biological function of modified nucleosides in tRNA and rRNA³. The dinucleosides were prepared by the phosphotriester approach⁴⁻⁵, which enabled us to prepare the desired compounds on a large scale (up to 1 g) in a relatively short time. Details of the synthesis and purification of ApU, m⁶ApU, m⁵ApU, i⁶ApU, UpA, Upm⁶A, Upm⁵A, and Upi⁶A will be published later. (We follow the nomenclature recommended by IUPAC-IUB⁶.)

The 270 MHz ¹H NMR spectra were recorded on a Bruker HDX-270 spectrometer, using D₂O lock, in FT mode. Interferograms were stored in 16K of computer memory and chemical shifts were measured on plots varying in scale from 60.38 Hz cm⁻¹ to 7.55 Hz cm⁻¹, to an accuracy of ±0.005 parts per million (p.p.m.). The shifts are measured relative to the methyl peak of a trace of 2,2-dimethyl-2-silapentane-sulphonic acid sodium salt (DSS) in D₂O, added as an internal reference. Owing to the relatively large line width (3.8 Hz) the coupling constants are accurate to only about ±0.3 Hz.

The spectrum of m⁶ApU was taken at 20° C, 0.05 M solution in D₂O. Further technical details will be published elsewhere (G. v. B. *et al.*, in preparation). Spin-spin ¹H decoupling experiments were carried out in the CW mode at 270 MHz. In addition, ¹H 100 MHz FT NMR spectra were recorded in our laboratories on a Varian HA-100 spectrometer at 4° C and at 30° C.

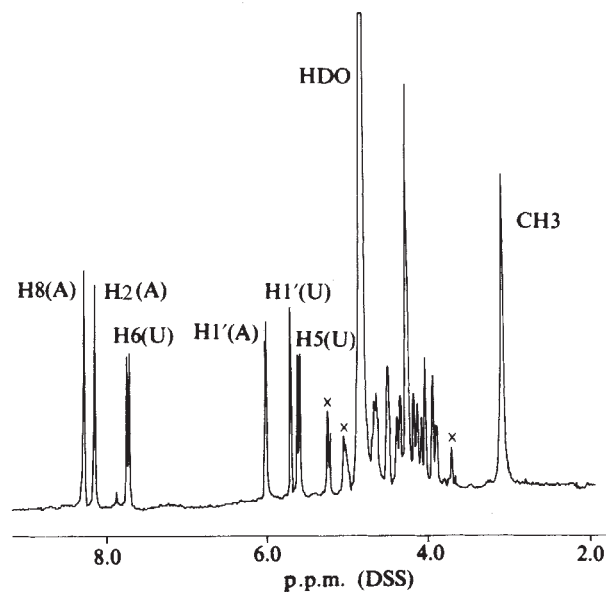


Fig. 2 270 MHz FT proton magnetic resonance spectrum at 20° C of 0.05 M solution of m⁶ApU. Crosses indicate resonances due to unidentified degradation products.

Figure 2 shows the entire ¹H spectrum including the straightforward assignment of the peaks due to the base protons and those due to the H1' protons. This low-field part of the spectrum is entirely analogous to that found in ApU⁷, in uridine⁸ (pU part) and in dimethyladenosine (C. A. and H. P. M. de Leeuw, unpublished) (m⁶A part). The tightly coupled ribose spectrum, consisting of ten proton signals (H2', H3', H4', H5', H5'' for each residue), spans a region of less than 1 p.p.m. and has hitherto defied all attempts at complete analysis. Even FT spectroscopy at 100 MHz proved ineffective. Indeed, it was recently stated⁹ that ¹³C NMR is of considerably more use in the case of polynucleotides, where the proton NMR spectra may well be totally unanalysable to yield ¹H-¹H and ¹H-³¹P coupling constants⁹. However, at 270 MHz the lines are well enough resolved to warrant a closer study. Figure 3a shows the final assignment of the observed transitions to the individual ribose protons. The decoupling experiments proved indispensable for this analysis, which is based on the following observations.

First, the two doublets at 6.02 p.p.m. and at 5.71 p.p.m. obviously belong to the H1' protons in the m⁶Ap and in the pU residues respectively, because in all dinucleoside monophosphates that contain adenosine and another base the H1' (A) signal invariably appears at the lower field position. In ApU the shift difference H1'(A)-H1'(U) equals 0.29 p.p.m.⁷, the corresponding difference in m⁶ApU amounts to 0.31 p.p.m. Now irradiation (decoupling) at 4.82 p.p.m., that is, in the region of the HDO signal, caused both the H1'(A) signal as well as the peak at 4.65 p.p.m. to simplify. This observation is positive evidence for the location of two more protons belonging to the m⁶Ap residue: H2'(A), obscured by the large HDO peak, and H3'(A) at 4.65 p.p.m. (Fig. 3). The latter signal shows a characteristic broadening (also found in the corresponding spectra of ApU and m⁵ApU examined by us) which we ascribe to the stereospecific ³J_{H-³¹P} coupling,

superimposed on the usual $J_{2/3}$ and $J_{3/4}$ pattern seen in mononucleosides.

Irradiation of H3'(A) caused changes in the line shape of the neighbouring peak occurring at 4.49 p.p.m., but, owing to the small (44 Hz) shift difference between the position irradiated and the peak observed, this result is not considered entirely conclusive. Irradiation at 4.49 p.p.m. (marked H4'(A) in Fig. 3), however, definitely caused a collapse of the fine splitting pattern of the high-field signals at 4.05 and 3.92 p.p.m.; irradiation at these latter positions in turn sharpened the 4.49 p.p.m. transition, thus providing a double check. The characteristic AB type quartet (4.05/3.92 p.p.m.) is easily identified as due to the H5' and H5'' protons of the m⁶Ap side chain. They appear within 0.06 p.p.m. from the corresponding H5',5'' quartet in dimethyladenosine (C. A. and H. P. M. de Leeuw, unpublished). Therefore, the signal of the H4'(A) proton, coupled to H3', H5' and H5'', is identified with the 4.49 p.p.m. resonance. This completes the assignment of all ribose protons contained in the m⁶Ap residue.

The pU protons could be assigned as follows: irradiation of the large peak at 4.26 p.p.m. caused the fine splitting of the H1'(U) signal to disappear, hence the H2'(U) proton signal is localised at or near 4.26 p.p.m. The intensity of this peak, however, corresponds to three protons, and we conclude that H3'(U) and H4'(U) accidentally resonate at approximately the same frequency as H2'(U). There remain two characteristic doublets, forming an AB quartet, flanking the 4.26 p.p.m. peak. These doublets are assigned to H5'(U) and H5''(U), shifted downfield (by 0.25–0.44 p.p.m.) with respect to the corresponding signals in uridine because of the attachment of the 5'-phosphate ester group.

Next, estimates of the chemical shifts and of a series of (first-order) vicinal coupling constants were obtained directly from the observed spectrum. Educated guesses for the few remaining 'H-H coupling constants ($J_{2/3}$, m⁶Ap residue; $J_{2/3}$, $J_{3/4}$; pU residue) were made based on the pseudorotation model of the ribose ring²; determination of the ¹H-³¹P coupling constants was aided by analysis of the line widths in conjunction with recently recorded values for these couplings at the 5'-position ($J_{5-P} = J_{5''-P} = 4.5$ Hz in 5'-adenosine monophosphate¹⁰) and in the 3'-position ($J_{3-P} = 8.0$ Hz in 3'-uridine monophosphate⁹) respectively. Unfortunately, such data on the 'reverse' monomers 3'-AMP and 5'-UMP seem to be lacking.

The computer program LAME was then used to simulate and plot the 270 MHz ribose spectra, assumed to consist of two independent seven-spin systems, to yield selfconsistent parameters, intensities, and line shapes matching those observed (Fig. 3b). The final parameters are given in Table 1. The relatively large line widths preclude the observation of any

small long range ¹H-¹H or ¹H-³¹P coupling so all these coupling constants were assumed to be zero.

Table 1 Chemical Shifts and Coupling Constants of m⁶ApU, 0.05 M Solution in D₂O at 20° C*

Residue	Proton designation	Chemical shift (p.p.m.)†	Coupling constants (Hz)‡
N ⁶ -mAp	H-1'	6.01	$J_{1'2'}$ 3.6
	H-2'	(4.82)§	$J_{2'3'}$ 5.0
	H-3'	4.65	$J_{3'4'}$ 5.7
	H-4'	4.49	$J_{4'5'}$ 2.3
	H-5'	4.05	$J_{4'5''}$ 3.0
	H-5''	3.92	$J_{5'5''}$ -13.1
	H-2	8.14	
	H-8	8.28	$J_{3'31P}$ 9.5
	CH ₃	3.07	
pU	H-1'	5.70	$J_{1'2'}$ 3.0
	H-2'	4.24	$J_{2'3'}$ 5.0
	H-3'	4.27	$J_{3'4'}$ 6.2
	H-4'	4.28	$J_{4'5'}$ 1.9
	H-5'	4.36	$J_{4'5''}$ 2.0
	H-5''	4.15	$J_{5'5''}$ -11.9
	H-5	5.60	J_{55} 8.3
	H-6	7.73	
			$J_{5'-31P}$ 3.5
			$J_{5''-31P}$ 3.5

* Data from 270 MHz Fourier transform spectra.

† The chemical shifts are measured relative to the methyl peak of DSS; the accuracy is of the order of 0.005 p.p.m.

‡ The coupling constants are accurate to about 0.3 Hz.

§ Estimated value. This transition is hidden under the HDO peak but irradiation at the position shown caused the H-1' (mA) doublet to collapse to a single peak.

|| Assumed values. Extensive overlap of the H-2', H-3' and H-4' transitions of the pU moiety precludes exact spectral analysis of these signals.

The accidental coalescence of the H2'(U), H3'(U), and H4'(U) signals in m⁶ApU proved to be a blessing in disguise. First, this feature allowed an unambiguous assignment of this ribose spectrum. Second, once this problem was unravelled, we were able to make a successful attack (unpublished) at solving the more complex patterns exhibited by the ribose spectra of ApU and of the dimethylated analogue m⁶ApU.

Figure 1 depicts the m⁶ApU molecule. Six torsional degrees of freedom, here denoted (ref. 11) ψ' , ψ , ϕ , ω , ω' and ϕ' , determine the exact folding of the sugar-phosphate backbone. Recent X-ray studies have disclosed fine details of this backbone folding in (base-base) stacked forms of ApU¹² and of GpC¹³ as they occur in the crystalline state. It is now of great interest to note that four backbone torsion angles can in principle be studied by NMR spectroscopy of dinucleoside phosphates in solution because vicinal coupling constants J_{HH} , J_{H31P} and J_{H13C} are torsion angle dependent. Unfortunately, the ω and ω' angles are not directly accessible from couplings without great experimental effort (for example, by measurement of ¹³C-¹⁷O couplings.)

We believe that the present NMR results are compatible with the X-ray structures of ApU and GpC with a few qualifications.

The coupling constant J_{HX} is related to the torsion angle τ_{HX} by the Karplus equation^{14,15}, which we write²:

$$J_{HX} = A_{HX} \cos^2 \tau - B_{HX} \cos \tau \quad (1)$$

From an analysis of X-ray and NMR data on nucleosides and nucleoside phosphates recently, best values for A_{HH} (10.5 Hz) and for B_{HH} (1.2 Hz) were extracted². Blackburn *et al.*¹⁶ proposed values for A_{HP} and B_{HP} of 16.3 Hz and 4.6 Hz respectively. Some care should be exercised, because in cases where mixtures of conformations (under dynamic equilibrium conditions) are present, one may in fact measure a time-average

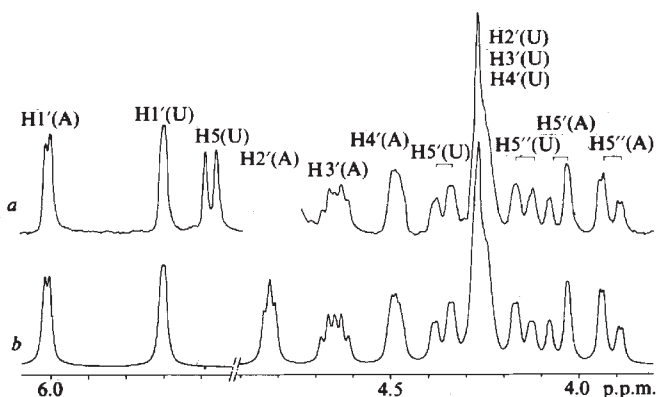


Fig. 3 a, parts of the spectrum of Fig. 2, eight times expanded. b, Computer simulated plot of the top spectrum. The H2' signal (far left) coincides with the HDO peak in the observed spectrum.

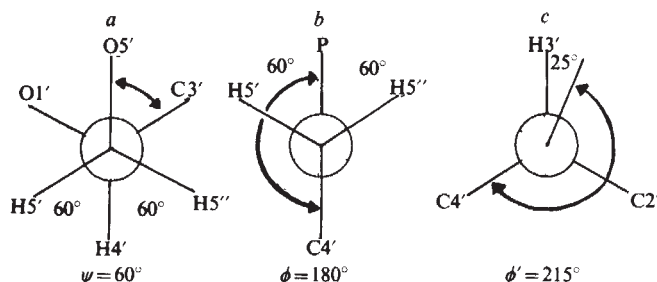


Fig. 4 a, Favoured rotamer about the C4'-C5' bond ($\psi = 60^\circ$); b, favoured rotamer about the C5'-O5' bond ($\phi = 180^\circ$); c, the favoured rotamer about the C3'-O3' bond ($\phi' = 215^\circ$). Backbone angles are indicated by arrows.

coupling that is related to the couplings of the individual conformers by:

$$J_{av} = J_I X_I + J_{II} X_{II} \quad (2)$$

Where X represents the mol fractions of each of the conformers present ($\sum X_i = 1$).

Thus, J_{av} is not directly related to the torsion angles of the individual forms. Keeping this limitation in mind (it plays a role in the interpretation of the couplings between the ribose protons), we see that the backbone torsion angles can be monitored as follows: ψ' by $J_{3/4}(U)$, ψ by $J_{4/5}(U)$ and $J_{4/5'}(U)$, ϕ by $J_{5/P}$ and $J_{5'/P}(U)$, ϕ' by $J_{3/P}(A)$ (Table 2). The torsion angles are defined¹¹ with respect to C, O or P atoms and not with respect to the hydrogens, but Newman projections of the classical rotamers will clarify the relationships (Fig. 4).

The two couplings related to ψ are small and practically equal; this is also true for the 1H - ^{31}P couplings related to ϕ . We feel safe to conclude that in each case a single rotamer predominates to an overwhelming extent. According to an equation proposed by Wood *et al.*¹⁷ the value of the sum $J_{4/5} + J_{4/5'} = 3.9$ Hz would correspond to a conformational purity of about 90% in favour of the *gg* ($\psi = 60^\circ$) rotamer. The Karplus parameters A_{HH} and B_{HH} derived by Altona and Sundaralingam² require a purity even closer to 100%. The sum $J_{5/P} + J_{5'/P} = 7$ Hz would correspond¹⁷ to a conformational purity of the $\phi = 180^\circ$ rotamer in excess of 90%. In fact, the present results extend, and at the same time slightly revise, the Σ/Σ' correlation line proposed by Wood *et al.*¹⁷.

The $J_{3/P}$ value could in theory correspond to four values of τ_{HH} : $\pm 25^\circ$ and $\pm 130^\circ$. The latter values are disregarded, because the corresponding ϕ' angles are clearly in a 'forbidden' region¹⁴. Our value of $\phi' = 215^\circ$ agrees surprisingly well with the X-ray results on ApU¹² and GpC¹³ (average $\phi' = 212^\circ$). The second possibility, $\phi' = 265^\circ$, has been observed to occur¹¹ in cytidine 3'-phosphate (orthorhombic) and cannot be excluded unambiguously from consideration. However, for the time being we prefer to postulate the existence of a single predominant rotamer about the C3'-O3' bond characterised by $\phi' = 210^\circ$ - 220° . This interpretation is consistent with the available X-ray studies on 3',5'-dinucleoside phosphates.

When we consider the ribose proton couplings, $J_{1/2}$ and $J_{3/4}$, of both residues in particular, a different situation emerges. At this point we wish to stress that the ribose couplings in m⁶ApU show perfectly 'normal' behaviour with respect to $J_{2/3}$ and to the sum $J_{1/2} + J_{3/4}$ as compared with purine and pyrimidine nucleosides where the base is known to occur predominantly in *anti* conformation². According to the proposed² multistate dynamic conformational equilibrium model of 3',5'-dinucleoside phosphates, the couplings $J_{1/2}$ (as well as $J_{3/4}$) are linearly related to the amount of stacked form present in the equilibrium and can be used (equation 2) to estimate this amount with reasonable accuracy (C. A., unpublished). Thus approximately 40% of the molecules of m⁶ApU occur in the well defined stacked conformer at 20° C (the

ribose rings occurring exclusively² as 3'-endo or N conformers, that is, exactly the ribose forms observed to occur in solid ApU¹² and GpC¹³). In the unstacked states the A and U ribose rings presumably do not affect each other appreciably and a normal 3'-endo/2'-endo (NS) conformational equilibrium is indicated for both residues.

Table 2 Interpretation of Observed Coupling Constants of m⁶ApU in Terms of Torsion Angles Along the Backbone

Coupling	Hz	τ_{HH}	Corresponding backbone angle	X-ray model ¹³
$J_{4/5'}(U)$	1.9	60°	ψ 60°	55°
$J_{4/5}(U)$	2.0	60°		
$J_{5'/P}(U)$	3.5	60°	ϕ 180°	170°
$J_{5/P}(U)$	3.5	60°		
$J_{3/P}(A)$	9.5	$+25^\circ$ -25°	ϕ' 265° ϕ' 215°	212°

(A) and (U) denote the adenosyl and uridyl residues respectively.

Finally, combining our findings regarding the extreme conformational preference along the C4'-C5', C5'-O5' and O3'-C3' bonds with the fact that both stacked and unstacked molecules are present in appreciable amounts, we conclude that these three backbone conformations under discussion do not change perceptibly on stacking or unstacking. Hence the conformation angles ψ , ϕ and ϕ' seem to have been prepared by nature in such a way that they fit both stacked (helical) and unstacked states. The same is true for the ψ' angle of the C3'-endo (N) ribose conformer. The main degrees of freedom available to these molecules on unstacking (aside from the ribose equilibria) must therefore be sought in the ω and/or the ω' angles. This hypothesis has already been put forth by several workers on the basis of known crystal structures^{18,19}, is in agreement with the results of all-valence molecular orbital calculations²⁰, and now confirmed by NMR spectroscopy in solution.

C. ALTONA
J. H. VAN BOOM
JENNY DE JAGER
H. J. KOENERS

Gorlaeus Laboratoria,
Leiden University

Department of Organic Chemistry,
Vrije Universiteit,
Brussels

G. VAN BINST

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Loss of methyl acceptor activity of ultraviolet-irradiated tRNA

THE features responsible for the recognition of specific sites for methylation by tRNA methylases are presently not understood. Studies by Kuchino and Nishimura suggest that a specific octanucleotide sequence is recognised by rat liver guanosine methylases¹. Furthermore, reports from Nishimura's laboratory show that a large (three-quarter) fragment from *E. coli* methionine tRNA could be methylated enzymatically². Shershneva *et al.*³, however have shown that fragments are not methylated until recombined. Studies in our laboratory have demonstrated that certain fragments from a mixture of *E. coli* tRNAs can serve as substrate for rat liver tRNA methylases. These fragments are believed to have an average S value of 0.64 and therefore would be about ten nucleotides long. Using ultraviolet-induced alterations, we have investigated further the importance of the molecular shape of tRNA as it affects its recognition by tRNA methylases.

E. coli tRNA was obtained from General Biochemicals, Chagrin Falls, Ohio. Aqueous solutions of *E. coli* tRNA (1 mg ml⁻¹) were irradiated at room temperature with an ultraviolet lamp (MR-4, 110 V, AC60 cycle, George W. Gates and Co. Franklin Square, Long Island) for varying times in quartz cuvettes 4 cm from the source. Control aliquots of the tRNA solutions were placed at the same temperature for the same time period. The tRNA samples were assayed by existing methods⁴ for their ability to be enzymatically methylated.

E. coli tRNA (10 mg) was incubated at 0°C for 20 min in 2 ml of 10 mM Tris buffer (pH 7.0) which contained 1 mM iodine and 0.5% (w/v) potassium iodide. The tRNA was precipitated with two volumes of ethanol and dialysed overnight against distilled water.

Ultraviolet irradiation of *E. coli* tRNA causes a marked decrease in its ability to serve as a substrate for tRNA methylases from rat liver (Fig. 1). The protective effect of magnesium towards maintaining conformation seems to be related to this effect since there was a tendency for 10 mM concentrations of magnesium to prevent the decrease in methylation. One of the primary alterations induced by ultraviolet irradiation of *E. coli* tRNA is the formation of a heterodimer formed between the nonadjacent residues 4-thiouridine and cytidine by 335 nm or 254 nm irradiation⁶. We therefore studied ultraviolet-induced alterations in 4-thiouridine in tRNA (Fig. 2). A possible correlation between the irradiation-induced changes in 4-thiouridine and the decreased capacity of the molecules to become methylated, can be examined by the effect of iodine on the methyl acceptor ability of tRNA. Iodine is a very selective reagent and only reacts with thiouridine residues⁷. Untreated *E. coli* tRNA (10 µg) gave values of $3,369 \pm 270$ c.p.m. when incubated with crude rat liver enzymes and S-adenosyl-(methyl-C¹⁴)-L-methionine. *E. coli* tRNA preparations, which had been treated with iodine for 10 min and had lost the 340 nm absorbance peak, had similar methyl incorporations ($3,449 \pm 223$ c.p.m. per 10 µg). Thus, the decrease in methylation due to ultraviolet irradiation of tRNA seems to be due to changes

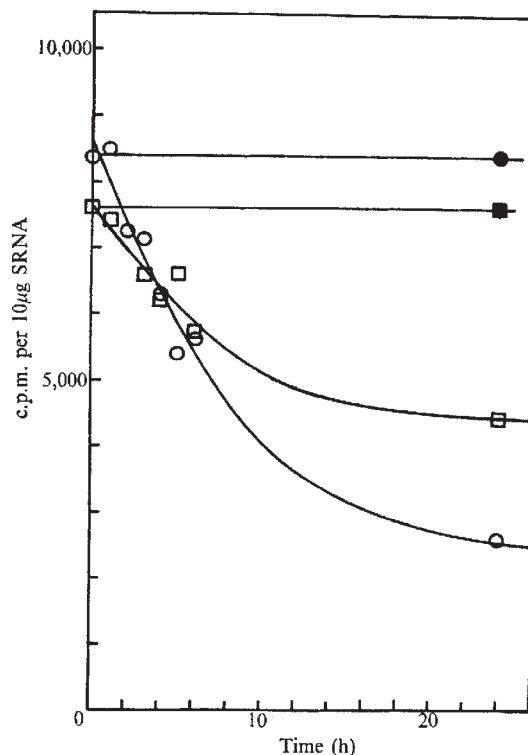


FIG. 1 Effect of ultraviolet irradiation on the methylation capacity of tRNA. Aqueous *E. coli* tRNA (3 ml, 1 mg ml⁻¹) with (□) or without (○) magnesium chloride (10 mM) were irradiated with an ultraviolet source as described in the text. The reaction mixture for the tRNA methylase assay, containing 20 µmol Tris buffer (pH 8.3), 10 µg aliquots of the particular tRNA preparation, 0.1 µCi of S-adenosyl-L-methionine (methyl-¹⁴C) specific activity 55.2 mCi mmol⁻¹, 600 µg protein equivalents of 100,000 g supernatant fraction of rat liver in a total volume of 0.25 ml, was incubated for 80 min at 45° C. Unirradiated controls with (■) or without (●) magnesium were maintained at the same temperature.

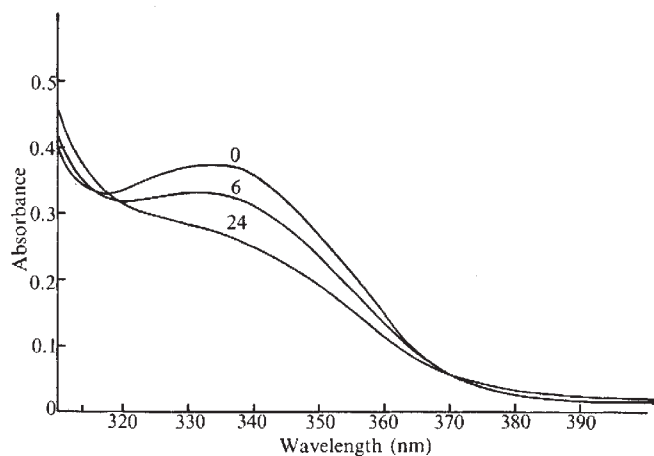


FIG. 2 Spectra of 4-thiouridine in the irradiated tRNA preparations used for methylation. Numbers on spectra refer to hours of ultraviolet irradiation as described in the text.

at some other locus in the tRNA molecule apart from thiouridine. The irradiation of tRNA has two major effects, one is the formation of U-U dimers and the other is the destruction of thiouridine residues. Since the loss in methyl acceptor activity could not be duplicated by iodine treatment, it is unlikely that the decrease in methyl acceptor activity is

due to the loss of thiouridine. Thus, it is more probable that the U-U dimer is important in the loss of the activity. This agrees with a theory that tRNA methylases require a uridine residue next to the methylation site to complex with the adenosine in the S-adenosyl-L-methionine (personal communication from J. Hilderheim).

Thus ultraviolet irradiation of *E. coli* tRNA produces a marked decrease in its efficiency to serve as a substrate for tRNA methylases from rat liver. Indirect preliminary evidence suggests that U-U dimer formation is the primary molecular alteration causing this decrease.

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P. I. FORRESTER
R. L. HANCOCK

Division of Medical Biochemistry,
Faculty of Medicine,
The University of Calgary,
Calgary 44, Alberta

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Ion Fluxes in Disk Membranes of Retinal Rod Outer Segments

THE process of transduction in vision is that mechanism whereby absorption of light by a photoreceptor cell in the retina results in a polarisation of the receptor cell membrane, excitation of the cell, and the eventual generation of a neural impulse to the brain from higher order neurones. It is already well known that the initial and primary process in vision is the absorption of photons by 11-cis retinal and concomitant isomerisation of the visual pigment to all-trans retinal. We have therefore studied events resulting from this photoisomerisation, events which provide a link between the absorption of light and the excitation of the receptor cell.

Yoshikami and Hagins¹ introduced a hypothesis which postulated that calcium ions were released from disk membranes of the retinal receptor cell and in turn blocked the flow of sodium ions across the plasma membrane of the cell, thereby leading to hyperpolarisation of the receptor. The implication of calcium in blocking the 'dark' current, or the movement of Na⁺ across the plasma membrane in the dark adapted state of the receptor cell, concerns us in the present paper, as well as the mechanisms whereby the disk membranes' supply of Ca²⁺ is renewed, the latter process presumably relying on an active uptake mechanism of some description. We report the results of experiments which suggest that the action of light ultimately induces release of Ca²⁺ from photoreceptor disk membranes of the vertebrate rod outer segment. We report evidence as well that these disk membranes possess an active mechanism for accumulating Ca²⁺.

If Ca²⁺ is released by the disk membrane, it must be able to replenish its supply of intradiskal calcium if the photo-

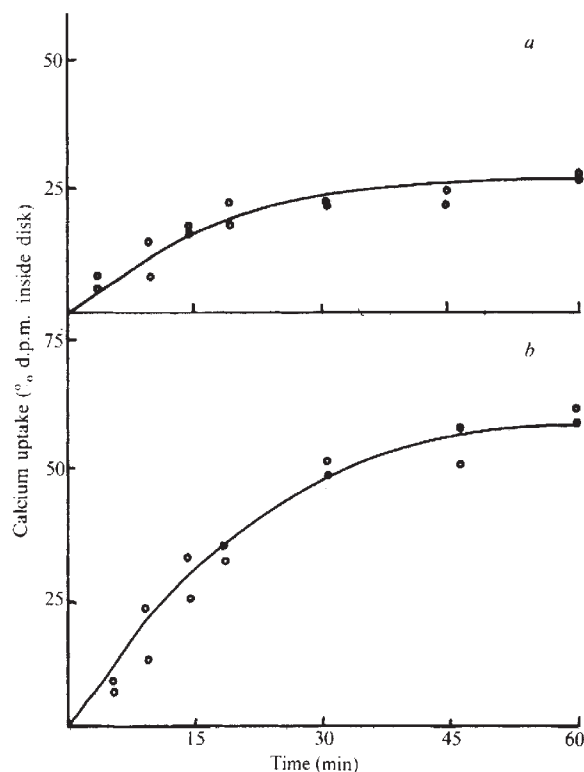


Fig. 1 Uptake of calcium by disk membranes *a*, in the dark; and *b*, in light. Membranes were incubated in 100 mM Tris buffer containing 10^{-5} M Ca²⁺ at 23° C

receptor is to recover its cell potential and be capable of repetitive hyperpolarisation. Such recovery of Ca²⁺ must inevitably depend on an active membrane ion pump which is capable of concentrating Ca²⁺ against a gradient. Were such a pump not present, the cell potential would not be maintained and the light-induced electrical response lost.

To investigate the presence of pump activity in the frog rod (*Rana catesbeiana*), we have developed a technique whereby specific ions or substances can be introduced into the intradisk or extradisk milieu of disk membrane vesicles. Retinae were gently dissected under red light and the rod outer segments shaken into 100 mM Tris buffer, pH 7.2. The crude outer segments obtained by this procedure were subsequently purified by the technique of Mason *et al.*². We then used the technique reported by Mason and Lee³ to make small vesicles of the photoreceptor membranes by sonication in an appropriate Ca²⁺-containing media to be described below, and subsequently incubating to allow membrane resealing. By this method, rods subjected to ultrasonic disruption for 15 min in 100 mM Tris buffer resealed in approximately 45 min. As evaluated by negative staining followed by electron microscopy, this treatment results in membrane sheets which resealed tightly to form vesicles of 800 Å diameter. The sonication temperature was controlled at $2^{\circ} \pm 3^{\circ}$ C by means of a special coolant cell. No protein denaturation was apparent as indicated by the absorbance at 500 nm (A_{500}) of the visual pigment. Furthermore, negligible leakage of Ca²⁺ was observed after resealing of the vesicles, that is, about 1–2% over a period of one hour.

In our studies of ion uptake and accumulation in the photoreceptor disk preparation, we used ⁴⁵Ca²⁺ in the extradisk space and studied Ca²⁺ uptake in the dark- and light-adapted photoreceptor membrane. Preliminary experiments on an isolated, intact disk membrane preparation and buffer-washed rods apparently yield results identical to the sonicated vesicle preparation with respect to the ability of disk membranes to accumulate Ca²⁺. Disk membranes from twenty retinae were suspended in 100 mM Tris buffer (pH 7.2) containing

10^{-5} M Ca^{2+} with 50 μl $^{45}\text{Ca}^{2+}$ tracer ($0.3417 \text{ mCi ml}^{-1}$) in a reaction volume of 5.0 ml. After various times up to 1 h of incubation in both dark and light, the suspension was made 0.25 mM in EGTA and centrifuged at $50,000g$ for 30 min. The supernatant was then taken off and the pellet solubilised in 5% cetyltrimethyl ammonium bromide (CTAB). Both pellet and supernatant were placed separately in 15 ml Brays solution and counted in a Picker-Nuclear scintillation detector.

The results of these experiments are given in Fig. 1. The disk membrane seems to contain an active pump which is capable of actively accumulating Ca^{2+} . Preliminary results indicate this pump is fuelled predominantly by an ATP-Mg system. If the disk membranes were examined after 30 min of incubation at 23°C , it was found that they had concentrated Ca^{2+} against a gradient, that is they had removed nearly 50% of the total Ca^{2+} from the extradisk solution. This result is highly significant because the disk membranes occupied at most 5% of the total suspension volume. Of further interest is the fact that the pump seems to be more active in fully light-adapted membranes (Fig. 1b). Bleached membranes incubated in the dark with $^{45}\text{Ca}^{2+}$ accumulate Ca^{2+} at a rate nearly three times that of dark-adapted membranes.

Ion fluxes in the vertebrate photoreceptor are difficult to study, particularly at the disk membrane level. The primary reason for this is the existence of a plasma membrane which surrounds the saccules, and this membrane is apparently capable of concealing intracellular ionic changes. Using a preparation of sonicated vesicles similar to that described above, we have examined light-induced fluxes of Ca^{2+} from the inside to the outside of disk fragments. We placed $^{45}\text{Ca}^{2+}$ inside disk membrane vesicles by sonication in 50 μl $^{45}\text{Ca}^{2+}$ and 10^{-4} M Ca^{2+} in 100 mM Tris and resealing. After 2 h, the preparation was exposed to light at 23°C in the presence of a 100 mM Tris buffer containing 0.25 mM EGTA for intervals ranging from 0–120 s. Addition of EGTA in this manner prevented pumping of Ca^{2+} back into the vesicles. At this point the membrane suspension was centrifuged as before, the supernatant removed, and the pellet dissolved in 5% CTAB. The samples were counted and corrected from counts to disintegrations min^{-1} . Before counting, the absorption spectrum of the solubilised pellet was taken on a Cary 14 spectrophotometer. By this technique, it was determined that after 2 min the visual pigment was completely bleached from native rhodopsin to metarhodopsin₃₈₀ II.

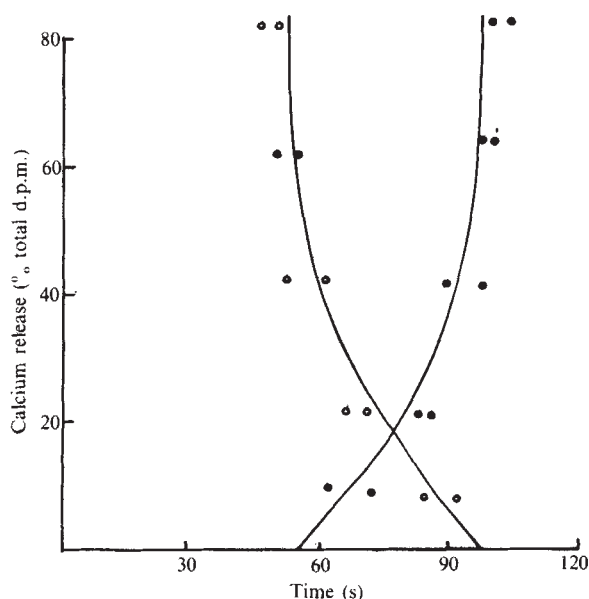


Fig. 2 Light-induced calcium release by sonicated bovine photoreceptor membranes over a time course of 120 s bleaching. Filled circles indicate percentage of Ca^{2+} in the extradisk space and open circles the percentage of Ca^{2+} in the intradisk space.

This experiment revealed that disk membranes release a large increment of Ca^{2+} upon light exposure (Fig. 2). Furthermore, these experiments seem to demonstrate that Ca^{2+} release is stepwise in response to rhodopsin photolysis, that is, upon partial bleaching of the membrane preparation, a certain amount of the intradisk Ca^{2+} is released, and no subsequent Ca^{2+} efflux occurs until further bleaching is effected.

We have also examined the stoichiometry of Ca^{2+} efflux relative to the extent of visual pigment bleaching. By determining the ΔA_{500} (difference in the absorbances of the partially bleached sample and the sample following full bleaching) of membrane samples solubilised in CTAB, one can calculate the number of mol of visual pigment bleached using an appropriate extinction coefficient (in this case, $41,000 \text{ cm}^{-1}$). Our initial experiments indicate that light-induced calcium efflux from the disk membrane is related to rhodopsin bleaching by a value close to unity; that is, the ratio of mol rhodopsin bleached to mol of Ca^{2+} released from a disk membrane vesicle is nearly one over a wide range of light intensities (from 5,000 photons per rod upwards to saturation and full bleaching).

The above preliminary results suggest three important properties of the disk membranes of rod outer segments: (1) they can actively accumulate Ca^{2+} in the dark and light, (2) the rate of accumulation is nearly three times greater in light than in dark, and (3) the disk membranes release Ca^{2+} as a consequence of light absorption and visual pigment bleaching. These results provide an initial experimental basis from which to pursue studies of the coupling of photon absorption and receptor cell excitation.

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W. T. MASON*
R. S. FAGER
E. W. ABRAHAMSON

Department of Chemistry,
Case Western Reserve University,
Cleveland,
Ohio 44106

Received July 31; revised November 27, 1973.

* Present address and address for reprint requests: The Physiological Laboratory, Downing Street, University of Cambridge, Cambridge CB2 3EG.

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Seasonal Changes in Human Plasma Levels of 25-Hydroxyvitamin D

THE relative importance of the two alternative sources of vitamin D, endogenous skin synthesis by the action of solar ultraviolet rays and dietary ingestion, is uncertain in Britain. In the past, classical rickets was common in springtime but rare in autumn^{1,2}, suggesting that summer sunshine was an important source of vitamin D. Seasonal variation in plasma antirachitic activity has been detected in more southerly latitudes in the United States, using bioassay techniques³, but data from Britain are scanty and some workers have considered the effects of sunshine to be minimal in this climate⁴. Possible age or sex differences in vitamin D nutritional status are also unknown.

Before it can act, vitamin D must first be converted to its 25-hydroxy derivative (25-OH-D) in the liver⁵, and this chief circulating metabolite can be measured by radio-stereo-assay, thus providing a precise index of vitamin D nutritional status⁶. Using this method, we have therefore determined plasma 25-

Table 1 Plasma 25-OH-D levels among 198 healthy white British subjects of both sexes sampled over a period of 22.5 month and grouped according to age and season

Population data			Season	Plasma 25-OH-D (ng ml ⁻¹)		Dietary vitamin D intake (IU d ⁻¹)	
Age range (yr)	Sex	Number		Mean	Range (95% confidence limits)	Mean	Range (95% confidence limits)
12 to 17	M only	59	Late September-early October 1971	22.6 ‡	13.4-37.9	147 (n=38)	43 to 495
7 to 10	Both	26	October-early November 1972	21.3	10.1-45.3		
18 to 37	Both	27	October-early November 1972	21.3	10.8-41.8	150 (n=19)	47 to 484
75 to 86	Both	18	Late November 1972-late January 1973	12.4 *	5.1-29.8		
14 to 17	F only	15	Late January 1973	12.9 *	7.2-23.2		
18 to 37	Both	19	Late March 1973	12.9 *	6.6-25.2		
14 to 17	F only	14	Early July 1973	17.0 †	7.6-38.4		
19 to 36	Both	20	Early August 1973	20.4	9.7-42.8		
Total		198					

* Difference from all other mean values (except †) $P < 0.001$; difference from † $P < 0.05$. † Difference from ‡ $P < 0.01$.

OH-D levels at different seasons over a total period of nearly 2 yr among healthy subjects of different ages. Results have shown a marked seasonal variation and we conclude that summer sunlight in Britain is a major determinant of vitamin D nutrition at the present time.

Healthy white subjects (198) of different ages and of both sexes were studied in 8 groups (Table 1). Young adults consisted of healthy students, academic and technical staff; children aged 7 to 17 were sampled from schools in the London area and they and their parents were informed volunteers; healthy geriatric subjects were statistically selected before participating in a nutritional survey in the London Borough of Camden. Plasma levels of calcium, phosphorus and alkaline phosphatase were also determined, and dietary vitamin D intakes were assessed from dietary tables in some of the school children and young adults. Plasma samples were deep-frozen before analysis for periods up to 22.5 months so that two or three populations were always compared in each assay. 25-OH-D seems to be very stable in human plasma because similar results were always obtained from replicate assays on the same specimens even after repeated freezing and thawing of the sample during the period of study. Most samples were analysed at least twice in separate assays, the coefficient of variation of replicate analysis in our hands being $\pm 10\%$.

Plasma 25-OH-D levels in the eight populations, grouped according to age and season, are shown in Fig. 1. A plot of cumulative frequencies indicated that their distribution was skewed. Mean levels after log transformation and reversion, with the normal range (95% confidence limits), are shown in Table 1. The three populations sampled in the winter of 1972-73 and spring 1973 were similar to each other and showed much lower mean 25-OH-D levels than either autumn groups of 1971 and 1972 or the summer group of August 1973. Levels rose from March to July 1973 and approached previous autumn values by August 1973. All ages showed similar values when sampled during the same season with the possible exception of the geriatric group; because sampling in this group began in late autumn a higher mean value might have been expected. Further investigation in elderly subjects is in progress. Comparison of plasma 25-OH-D levels in boys at each year of age from 12 to 17 showed no change during puberty. Plasma alkaline phosphatase levels rise during the puberty growth spurt⁷ but there was no evidence that this 'flare' is associated with relative vitamin D deficiency, and we have previously reported the lack of correlation between individual plasma levels of alkaline phosphatase and 25-OH-D⁸. A sex difference was found in adults in August 1973 when females showed significantly higher levels than males ($P < 0.01$, see Fig. 1). An apparent tendency towards this sex difference at other times was not significant. Although a physiological difference between sexes in this respect cannot be excluded, a simpler reason may be that a summer dress gives greater skin exposure than does a shirt and trousers.

Dietary vitamin D intakes, in those subjects in whom it was assessed, are shown in the table. Five of the thirty-eight school boys gave intakes of less than 70 IU daily but all subjects showed normal plasma levels of calcium, phosphorus and alkaline phosphatase. No significant correlation was observed between plasma 25-OH-D and dietary vitamin D intake in either population (correlation coefficient $r = 0.04$ for both groups) or between 25-OH-D and plasma calcium levels in any group (maximum correlation coefficient $r = 0.228$, $t = 1.765$, $0.1 > P > 0.05$).

Our finding of a prominent seasonal variation in plasma 25-OH-D levels indicates that contrary to some views⁴, summer sunlight is an important, and possibly at present the chief, determinant of vitamin D nutrition in Britain. The skewed distribution of plasma 25-OH-D presumably arises from minority participation in outdoor activity or sunny holidays; for example, much higher summer levels of 25-OH-D have been reported in the United States among lifeguards whose exposure to sunshine is particularly high⁶. The low dietary vitamin D intakes found in some healthy subjects and the somewhat surprising absence of significant correlation between intake and plasma 25-OH-D in autumn are further evidence

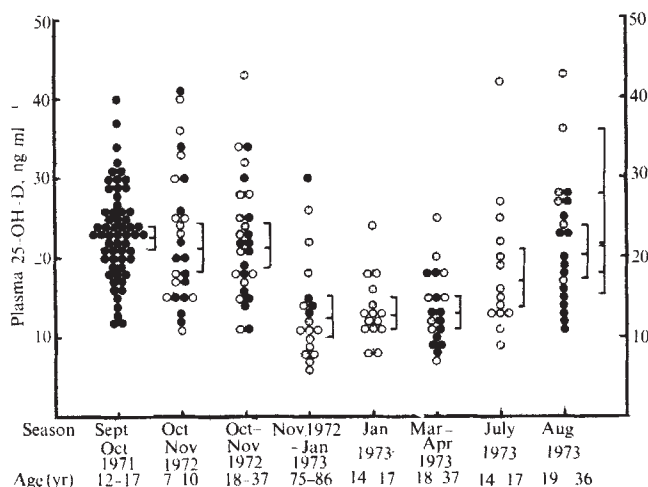


Fig. 1 Plasma 25-OH-D levels among 198 healthy white British subjects of both sexes grouped according to age and season. Bars indicate the mean and limits of $2 \times \text{s.e.}$ after log transformation and reversion; values in August 1973 are shown for males and females both separately and together. ●, Males; ○, females.

for the greater contribution of summer sunlight than that of diet to nutritional status. In the United States, foods are widely fortified with the synthetic compound ergocalciferol (vitamin D₂) which undergoes 25-hydroxylation in the same manner as the natural vitamin cholecalciferol (vitamin D₃); both metabolites are measured equally by the assay⁹. Haddad has recently separated both 25-hydroxy-derivatives in human plasma in the United States and found the major constituent to be 25-hydroxycholecalciferol, strongly suggesting that skin synthesis in that climate plays a more important role than dietary ingestion¹⁰. Although we had no reason in the present study to suppose that the actual choice of diet, with regard to vitamin D content, changes with the seasons, it is still possible that part of the seasonal variation in plasma 25-OH-D may result from a natural increase in the vitamin D content of certain foods during summer.

Although the concentrations of many plasma constituents vary with age and between the sexes, prominent seasonal variation of an important constituent has not previously been documented in tables of normal values. Present data are of clear clinical importance; rickets from whatever cause may take months or years to develop and if it is first diagnosed in autumn when early healing may be evident¹¹, the existence of vitamin D deficiency may only be recognised if the relatively high normal range for 25-OH-D in autumn is appreciated. Similar problems apply to the interpretation of surveys in populations at risk such as the immigrant Asian community in Britain.

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T. C. B. STAMP
J. M. ROUND

Department of Human Metabolism,
University College Hospital and Medical School,
London WC1

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Gas Vapour Phase Constituents and SH Reactivity of Cigarette Smoke Influence Lung Cultures

CELL cultures exposed to the gas vapour phase of cigarette smoke have been reported to display essentially the same alterations as those exposed to whole cigarette smoke¹⁻⁴. Furthermore, animals inhaling either whole smoke or the gas vapour phase of cigarette smoke have been reported to show increased pulmonary carcinogenesis^{5,6} and immunosuppression⁶⁻⁸.

In view of the widely accepted concept that it is predominantly the particulate matter, the 'tar', of cigarette smoke, which is responsible for lung cancer in man⁹, the exploration of the biological effect of the gas vapour phase of cigarette smoke

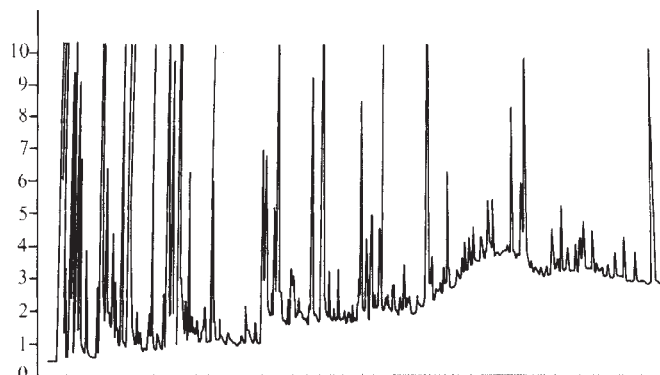


Fig. 1 Gas chromatogram of cigarette smoke without a charcoal filter.

seems warranted. We report here a study designed to compare effects of two types of cigarette smoke, containing the same amount of particulate matter but differing in their content of gas vapour phase constituents, on hamster lung cultures.

The cigarettes were made of commercial tobacco, had a weight of 1,020 mg per cigarette, a length of 80 mm, a diameter of 8 mm, with a paper porosity of 25 ml cm⁻² mm⁻¹, and had a white filter of cellulose acetate, length 18 mm. The lung cultures were prepared from golden hamsters 3 to 5 d old and exposed under standardised conditions in a Filtrona CFM 12 smoking machine to fresh puffs of cigarette smoke². Before exposure, the cigarettes were placed in a glass container 3.5 cm long and 0.9 cm wide. To reduce the gas vapour phase content in one type of cigarette, 230 mg of activated carbon was placed in the glass holder. The activated charcoal was prepared from coconut shell, had an active surface of 1,200 m² g⁻¹, a CCl₄ activity of 65%, and a granulation (Tyler mesh) of 32. Special care was taken that the freshly activated charcoal was placed in the glass container immediately before each exposure, otherwise, as mentioned previously¹⁰, the activated charcoal becomes saturated with vapour and loses its activity.

Eight different experiments were carried out, comprising nearly 3,000 hamster lung cultures. For each experiment, a minimum of twelve sets of cultures consisting of thirty-six

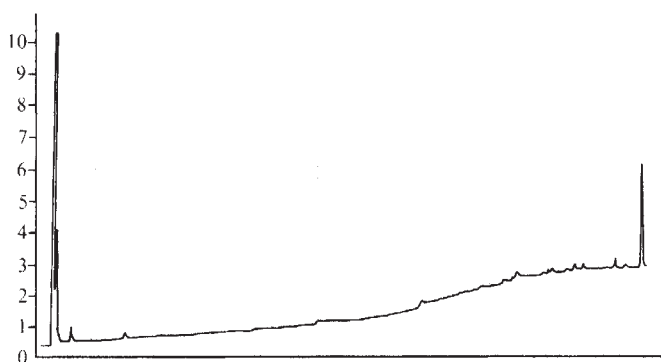


Fig. 2 Gas chromatogram of cigarette smoke with a charcoal filter.

cover-slips and twelve bottles with primary matched lung cultures was used. The sets comprised control cultures not exposed to cigarette smoke, cultures exposed to three puffs per day (25 ml at intervals of 58 s) of fresh smoke from cigarettes without a charcoal filter for 3 consecutive days, and cultures exposed in the same manner to four to six puffs from cigarettes with a charcoal filter. It was necessary to give the smaller

Table 1 Comparison between the effects of fresh cigarette smoke with a high or low content of gas vapour phase and SH reacting constituents on hamster lung cultures

Type of cigarette	Characteristics of smoke				Number of exposures (puffs per exposure)	Morphological and cytochemical alterations				
	Nicotine (mg per cigarette)	Total particulate matter (mg per cigarette)	Amount of gas vapour phase	SH-index (%)		Cyto-toxicity	Stage I Loss of macrophages	Stage II Mitosis Exposed Control	Abnormalities of: Prolifera- tion	DNA content in chromosomes ($n=1,200$)
Commercial tobacco	1.4	25	++++	44	3 to 20 (3)	++++	++++ to +++++	3.7 ± 0.4 pCo 0.005	++++	++++
Commercial tobacco + charcoal filter	1.6	22	+	7	3 to 20 (4 to 6)	+	+	1.3 ± 0.2	to ++	to ++
None (control)	—	—	—	—	—	—	—	—	+	+

+ to ++, Slight to moderate; +++ to +++++, pronounced; n, Number of metaphases and telophases measured.

* Doubtful.

number of puffs from cigarettes without charcoal filters, because of their marked cytotoxic effects on the cultures. Subcultures were prepared from controls and exposed cultures

once a week, and the latter were exposed again to smoke as described above. Media were changed either immediately or 5 min after exposure.

In view of the importance of SH groups in cell metabolism and division¹¹, the smoke of both types of cigarettes was examined for constituents reacting with SH groups. The SH index of the smoke was determined in the following manner: sixty cigarettes were smoked in a rotary smoking machine, according to CORESTA standards. The continuous stream of smoke was passed through a 1 l glass flask which was rotated in a water bath at 40° C. The flask contained 5 ml of a 0.1 M solution of cysteine hydrochloride in water. After the exposure to smoke, the content of the flask was transferred to a titration vessel, and the remaining cysteine which had not reacted with the smoke was titrated with copper sulphate. The results were expressed as the percentage of cysteine consumed by the smoke of sixty cigarettes (SH index).

The sequential alterations were studied in live cultures under phase contrast microscopy and in the original stained cultures from 1d to 8 months after exposure. The frequency of mitotic abnormalities was obtained by counting all abnormal mitoses and relating them to the total number of mitoses in each culture. DNA determinations were carried out by Feulgen microfluorometry² on the metaphases and telophases of the original stained lung cultures.

Gas chromatograms of smoke from cigarettes with a charcoal filter displayed a striking reduction of the gas vapour phase constituents and of the SH index, whereas the content of nicotine and total particulate matter closely resembled that of the smoke from cigarettes without a charcoal filter (Table 1, Figs 1 and 2). Both types of smoke produced results in all cultures, although the degree of response to cigarette smoke without a charcoal filter was not always the same for primary lung cultures prepared from different hamsters.

There were significant differences between control cultures and those exposed to smoke from cigarettes without a charcoal filter. From 1 to 4 d after exposure, marked cytotoxic effects, such as pycnosis, necrosis, cell death and a striking reduction of alveolar macrophages were observed in the cultures (Table 1). This cytotoxic effect was followed by atypical growth, a high frequency of mitotic abnormalities, and alterations in the DNA content of the chromosomes (Table 1, Figs 3a-e, 4 and 5). These atypical proliferative alterations were essentially the same as those observed in human lung cultures exposed to smoke from Kentucky Standard and Marijuana cigarettes^{12,13}. Cultures exposed to smoke from cigarettes with a charcoal filter showed much milder changes of all these features, and thus resembled more closely the control cultures (Table 1 and Fig. 3a-e). Macrophages were observed in both young and older cultures (Fig. 3e). The differences in the DNA complement of chromosomes between control cultures and cultures exposed to cigarette smoke with and without a charcoal filter are

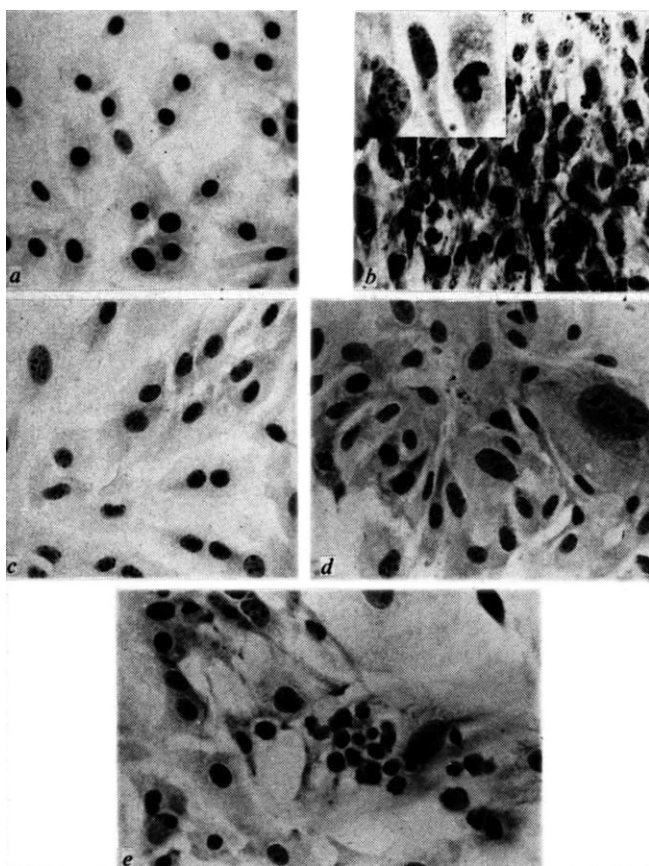


Fig. 3 *a*, Fibroblastic cells in monolayer of a 186 d control hamster lung culture stained with haematoxylin and eosin (H and E) ($\times 178.50$). Note well separated cells. *b*, The same culture as in *a*, but 26 d after sixteen exposures to three puffs each of fresh cigarette smoke without a charcoal filter. (H and E, $\times 178.50$). Note irregularity of growth, abnormal mitosis and irregular shape of nucleus (inset $\times 382.50$). *c*, The same culture as in *a*, but in 26 d after sixteen exposures to six puffs each of fresh cigarette smoke with a charcoal filter (H and E, $\times 178.50$). Note similarity of culture with control culture. *d*, Fibroblastic cells of a 77 d hamster lung culture, 66 d after three exposures to three puffs each of fresh cigarette smoke without a charcoal filter (H and E, $\times 191.25$). Note absence of macrophages, irregular sizes of nuclei and abnormal mitosis. *e*, The same culture as *d*, but 66 d after three exposures to six puffs each of fresh cigarette smoke with charcoal filter (H and E, $\times 191.25$). Note presence of macrophages.

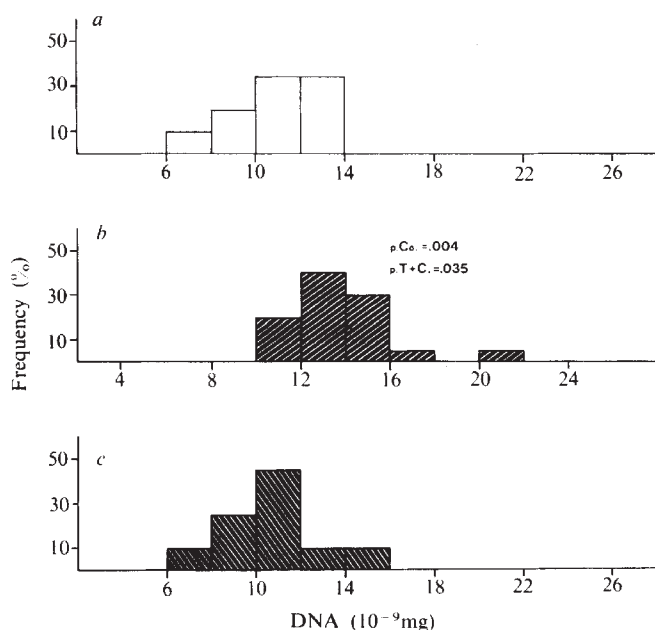


Fig. 4 DNA content (Feulgen microfluorometry) in metaphases of fibroblastic cells ($n=60$) from, *a*, 140 d control hamster lung culture; *b*, 132 d after six exposures to three puffs each of fresh cigarette smoke without a charcoal filter, and, *c*, to four puffs each with a charcoal filter.

illustrated in Figs 4 and 5. Although after exposure to smoke from cigarettes without charcoal the DNA content in metaphase and telophase was significantly greater and showed more variability than that of controls, there were no significant differences in the DNA content of cultures exposed to smoke from cigarettes with charcoal.

It seems, therefore, that the exposure of hamster lung cultures to a cigarette smoke with reduced gas vapour phase constituents

and low SH reactivity not only produces less damage to the cells, but also less abnormal growth and disturbance of DNA complement in the chromosomes than smoke with greater amounts of gas vapour phase constituents and a higher SH reactivity. The observation, that these differences were found although both types of smoke had essentially the same amount of particulate matter (Table 1), indicates that gas vapour phase constituents contributed to the abnormalities. It has been reported that cigarette smoke inhibits SH-dependent enzyme systems¹⁴⁻¹⁶, and that free radicals are responsible for this phenomenon¹⁷. The finding that free radicals are present in the gas vapour phase of cigarette smoke¹⁸, not only supports the concept of the importance of gas vapour phase constituents for abnormal growth¹⁻⁶, but it also indicates that a disturbance of SH equilibrium in cells and tissues may be one of the steps in the sequential changes. On the other hand the observation that, even after the reduction of gas vapour phase components and of SH reactivity, the hamster lung cultures still showed some alterations, indicates the contribution of particulate matter of cigarette smoke to the occurrence of cellular abnormalities. In other words, the reduction of either gas vapour phase alone or of particulate matter alone does not yield a smoke which is harmless to cells in lung cultures.

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CECILE LEUCHTENBERGER
RUDOLF LEUCHTENBERGER
IRENE ZBINDEN

Swiss Institute for Experimental
Cancer Research,
Bugnon 21,
Lausanne

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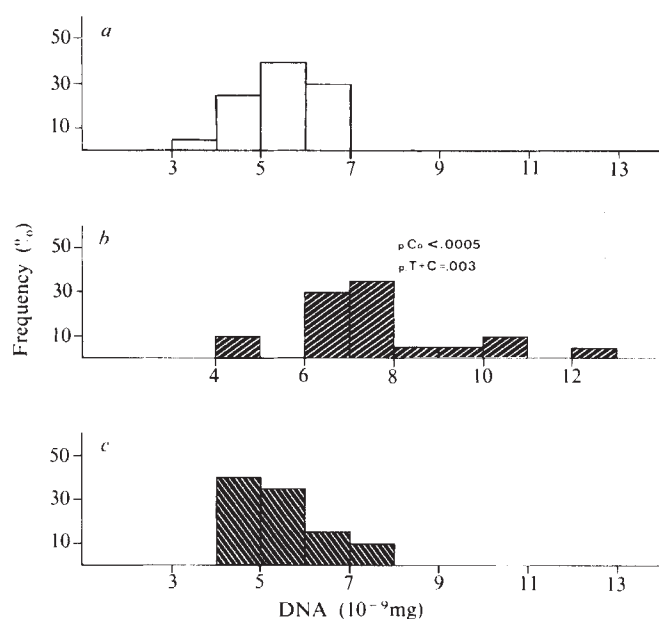


Fig. 5 DNA content (Feulgen microfluorometry) in telophases of fibroblastic cells ($n=60$) from, *a*, 168 d control hamster lung culture; *b*, 120 d after fourteen exposures to three puffs each of fresh cigarette smoke without a charcoal filter and, *c*, to four puffs each with a charcoal filter.

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Poikilothermia and Susceptibility of Suckling Mice to Coxsackie B1 Virus

INFECTIONS with most types of Coxsackie virus are fatal to newborn mice in normal conditions. This susceptibility decreases sharply with age and, when the mice are a few days old, the infection runs a subclinical course¹. As yet no satisfactory explanation of this phenomenon has been offered.

A greater susceptibility to some virus infections occurs if the temperature of the host is reduced and adult mice which are made hypothermic become susceptible to Coxsackie virus².

In most newborn animals there is a physiological hypothermia and poikilothermia³. We therefore studied the possible influence of body temperature on the susceptibility of newborn mice to Coxsackie virus.

The body temperature was measured in suckling mice varying in age from 12 h to 10 d. The mice were kept in cages held at room temperature (22 to 24°C). The body temperature was measured in the peritoneal cavity by insertion of a thermister. Each point in Fig. 1 indicates the average temperature of thirty to fifty suckling mice. The temperature was measured of mice taken directly from the nest with the mother on the nest, and it was ensured that she had stayed there for a period of at least 10 min. The temperatures measured 10 min and 20 min after the mother had been taken away from the nest are also shown. The babies were left piled just as when the mother normally leaves the nest. Each mouse was used for measuring one temperature only.

As the influence of poikilothermia on the course of infection is obviously related to the behaviour of the mothers, this was followed for a total of 6,360 min; twenty-two litters for 180 min in daylight and twenty litters for 120 min in twilight. The mothers were found to be away from the nest for an average total of 49% of the time (55% in twilight and 45% in daylight). Absences for periods longer than 10, 20 and 30 min, respectively, were recorded as 42%, 31% and 20% of the time. Concerning these longer absences, no

significant differences could be found between daylight and twilight.

The hypothermia and, particularly, the poikilothermia found in newborn mice thus decreased within a few days. At an age of 9 to 10 d, the mice had a temperature, with their mother on the nest, just as high as adult mice. The poikilothermia decreased especially after about 6 d. The effect of this decrease was pronounced, particularly when the mothers were kept away for relatively long periods, and is in agreement with previous results⁴.

The mortality of suckling mice caused by Coxsackie B1 virus in relation to the age at which the mice were infected was also studied and is given in Fig. 1. The mice were infected intraperitoneally with a dose of 60 LD₅₀ (titrated intracerebrally in 1-d-old mice). An abrupt decrease in mortality occurred around day 6, and after day 8 no mortality was seen with this dose of virus. This result is also in agreement with a previous report⁵.

Table 1 Results of various experiments in which infected and uninfected newborn mice were placed at ambient temperatures of 22 to 24°C and 34°C.

Experiment No.	Age (d)	Virus dose	Inoculation Route	Mortality at ambient temperature	
				22 to 24°C	34°C
1	1.5	60 × LD ₅₀	Intracerebral	6/7	1/7
2	0.5	60 × LD ₅₀	Intracerebral	6/7	3/7
3	1.5	60 × LD ₅₀	Subcutaneous	7/7	1/7
4	2	60 × LD ₅₀	Intraperitoneal	12/12	1/12
5	2	60 × LD ₅₀	Intraperitoneal	10/10	3/20
6	1.5	60 × LD ₅₀	Intraperitoneal	5/5	1/5
7	3	60 × LD ₅₀	Intraperitoneal	10/10	3/25
8	3	No virus	—	—	0/20
9	1.5	No virus	Phosphate buffered saline (intraperitoneal)	—	4/20

To study the mortality of newborn mice in which the body temperature was stabilised around the lowest body temperature of the baby mice which survived, that is, 9 to 10-d-old babies, the cages with the mothers and their 1 to 3-d-old babies infected with a dose of 60 LD₅₀ Coxsackie B1 virus were placed in an incubator at 34°C. The average body temperature of newborn mice kept in these conditions was found to be 35.8°C throughout the experiment. At this temperature, the suckling mice were lying free in the cage and were never found nesting. Table 1 shows the results of various experiments which revealed a pronounced reduction in mortality in mice with this body temperature throughout the course of infection.

Thus a correlation between poikilothermia and susceptibility to Coxsackie B1 virus was found. The higher temperature in the older mice might inhibit viral infection by decreasing virus replication in infected cells or by potentiating host defence mechanisms. Obviously the destruction of cells by growth of virus may also be different in hypothermic and normothermic conditions. Further evaluation of these possibilities is in progress in this laboratory.

BØRGE TEISNER
SVEN HAAHR

Institute of Medical Microbiology,
University of Aarhus,
8000 Aarhus C

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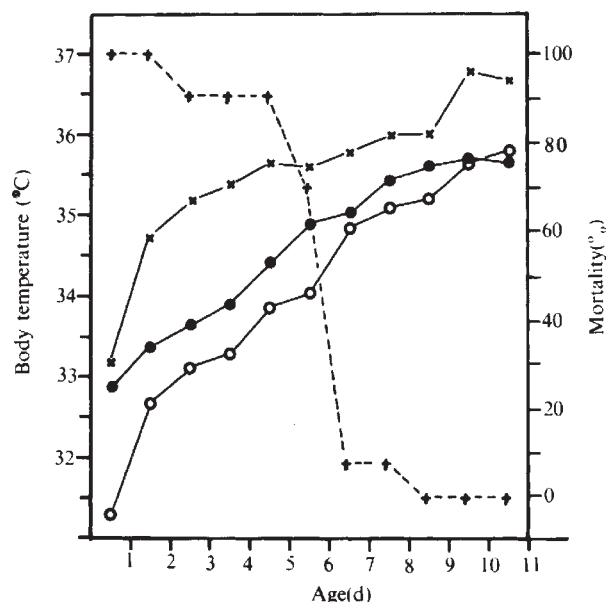


Fig. 1 Body temperature in suckling mice of varying age. The temperature was measured on mice taken directly from the nest with the mother on the nest, and it was ensured that she had stayed there for at least 10 min (×). The temperatures measured 10 min (●) and 20 min (○) after the mother had been taken away from the nest. The mortality in relation to the age at which the mice were infected (+).

Interaction of Mercurials with Salmon Serum Lipoproteins

ALTHOUGH the toxic effects of mercury and its derivatives are well known¹⁻⁷ relatively little is known about the transport mechanisms involved in its distribution throughout the body. One possible means could be the serum lipoproteins through reactions involving lipids and proteins. Various metal ions bind with phospholipids⁸; for example, calcium ions bind to anionic sites of phospholipids in lipid monolayers⁹. In addition, phospholipids provide negatively charged sites for calcium ion exchanges in biomembranes¹⁰ and contribute to the transport of ferrous ions in biphasic systems¹¹. These findings, together with the fact that mercurials bind with negative sites of proteins, led us to explore the possibility that mercurials are actively sequestered by the serum lipoproteins.

We have now demonstrated that inorganic mercury ($^{203}\text{HgCl}_2$) and organic mercury ($\text{CH}_3^{203}\text{HgCl}$) compounds readily bind with the major classes of serum lipoproteins of sockeye salmon (*Oncorhynchus nerka*). The role played by lipid in these interactions is discussed here in reference to data obtained with a CHCl_3 : H_2O model system.

Blood was drawn with a 3 ml syringe from the caudal vein of juvenile salmon 12–15 h after feeding. The serum was obtained by centrifuging the blood for 5 min at 1,500g to sediment the red cells. EDTA was added to the serum to give a concentration of 5 mg per 100 ml. The lipoprotein density classes were obtained by ultracentrifugation¹². The lipoproteins (VLDL, LDL and HDL) and their apoproteins¹³ were dialysed exhaustively against 0.01 Tris buffer (pH = 7.2) containing 0.15 M NaCl and 0.005 M NaN_3 . VLDL is very low density lipoprotein of $d < 1.006 \text{ ml}^{-1}$; LDL is low density lipoprotein of $d = 1.006 - 1.063$; HDL is high density lipoprotein of $d = 1.063$ to 1.21 g ml^{-1} . Apo VLDL, apo LDL and apo HDL are delipidated apoproteins of VLDL, LDL and HDL. The lipoproteins and the apoproteins were diluted to volume so that the final concentrations approximated those present in the serum (Table 1). Protein was determined by the method of Lowry¹⁴ and thin-layer chromatography was used to separate lipids^{15,16} isolated from the lipoproteins. The protein:lipid ratios of the lipoproteins were as follows (w/w): VLDL (9:91), LDL (17:83) and HDL (46:54). The phospholipids in the VLDL, LDL and HDL represented 17, 36, and 55% of the total lipids, respectively.

The incorporation of the mercurials into the lipoproteins and apoproteins was examined at 10° C by placing 5.0 ml of lipoprotein or apoprotein solution (Table 1) inside a dialysis bag (4,000–6,000 molecular weight unit pore size) containing 0.1 ml of $15.07 \mu\text{M } ^{203}\text{Hg}^{2+}$ or $\text{CH}_3^{203}\text{Hg}^+$. The final concentration of the mercurials was $3.07 \times 10^{-1} \mu\text{M}$ 0.06 p.p.m. The levels of Hg in *O. nerka* were shown to vary from 0.01–0.1 p.p.m.

(private communication with E. J. Gauglitz jun.). The bag was then placed in 50 ml of buffer solution containing the same concentration of mercurial. The top of the bag was pressed shut with a rubber stopper against the neck of the flask and the solution was stirred continuously. Aliquots (1 ml) were taken from inside the bag and from the surrounding medium. Each aliquot was assayed for radioactivity and the differences between the d.p.m. ml^{-1} inside and outside represented the ^{203}Hg associated with the lipoproteins and apoproteins. After maximum incorporations, the solutions were transferred into fresh bags which were placed into 125 ml suction flasks containing Hg-free buffer. Fresh buffer flowed into the system at a rate of 1.0 ml min^{-1} and the excess was allowed to flow out of the side arm. The release of ^{203}Hg by the lipoproteins and apoproteins was determined as described for the incorporation studies. No evidence was found for amino acid residues¹⁴ or phospholipids¹⁵⁻¹⁷ outside the membrane that could possibly bind with the mercurials released from the lipoproteins.

The role of lipids in sequestering the mercurials was examined with a CHCl_3 : H_2O system. The incorporation of $^{203}\text{Hg}^{2+}$ and $\text{CH}_3^{203}\text{Hg}^+$ was followed by placing 1.5 ml of fresh glass distilled CHCl_3 containing $1.25 \mu\text{mol}$ of lipid in a glass centrifuge tube equipped with a 'Teflon'-lined screw cap. Then 1.0 ml of buffer solution containing $3.07 \times 10^{-4} \mu\text{mol}$ of radioactive metal ion was added. The solution was shaken vigorously for 30 s and then shaken in a water bath (14° C) for 2.0 h. The tubes were centrifuged for 15 min at 4,000 r.p.m. in a clinical centrifuge. Aliquots were taken from both phases and assayed for ^{203}Hg . The CHCl_3 phase was then extracted with 1.0 ml aliquots of buffer solution to determine whether mercuric or methylmercuric ions were released into the aqueous phase. The presence of radioactive ions in the aqueous phase would indicate whether the metal-lipid complexes were readily dissociable. Duplicate runs were conducted in each experiment and the results were comparable within an error of $\pm 3\%$.

The partitionings between the CHCl_3 and the buffer solutions were repeated with solutions containing Hg^{2+} and ^{14}C -labelled phospholipids to determine whether the metal-phospholipid complexes entered the aqueous phase. It was shown that no more than 2% of the ^{14}C was associated with the aqueous phase in each extraction. Thus, the Hg-phospholipid complexes were not removed from the CHCl_3 to a significant extent.

Lipids of high purity (>99%) from commercial sources were analysed by thin-layer chromatography^{15,16}. No impurities were found. Phospholipids—phosphatidyl choline (PC), phosphatidyl ethanolamine (PE) and phosphatidyl serine (PS)—were from bovine sources. The mercurials were obtained from International Chemical and Nuclear Corp., Irvine, California.

Evidence is presented to show that mercuric and methylmercuric ions are sequestered by the VLDL, LDL and HDL of fish serum under physiological conditions (Table 1). The fact

Table 1 Incorporation of Mercurials into Serum Lipoproteins of Salmon (*O. nerka*)

Mercurial	Lipoprotein density class	Mg lipoprotein ml^{-1}	Mg protein ml^{-1}	% Incorporation (maximum)	% Released *
$^{203}\text{HgCl}_2$	VLDL	1.67	0.16	22	42
	apo VLDL		0.17	2.7	21
	LDL	2.46	0.40	27	50
	apo LDL		0.40	5.9	39
$\text{CH}_3^{203}\text{HgCl}$	HDL	2.38	1.04	22	23
	apo HDL		1.06	25	2.8
	VLDL	1.67	0.16	11	28
	apo VLDL		0.17	3.3	39
$\text{CH}_3^{203}\text{HgCl}$	LDL	2.46	0.40	13	46
	apo LDL		0.40	5.2	27
	HDL	2.38	1.04	35	38
	apo HDL		1.06	35	38

The procedure is described in text.

* Percentage released in a 24 h period from the maximum incorporation obtained.

Table 2 Interaction of $^{203}\text{HgCl}_2$ and $\text{CH}_3^{203}\text{HgCl}$ with Lipids: Metal-Lipid Complexes Formed in CHCl_3 Solution

Lipids	Lipid in CHCl_3 (μmol)	Mercurial in aqueous phase (μmol)	Metal lipid complexes formed in CHCl_3 phase ($\mu\text{mol } ^{203}\text{Hg}^{2+}$)	Metal lipid complexes formed in CHCl_3 phase ($\mu\text{mol } \text{CH}_3^{203}\text{Hg}^+$)
Phosphatidyl choline	1.25	3.07×10^{-4}	1.4×10^{-4}	$< 0.02 \times 10^{-4}$
Phosphatidyl ethanolamine	1.25	3.07×10^{-4}	1.6×10^{-4}	$< 0.02 \times 10^{-4}$
Phosphatidyl serine	1.25	3.07×10^{-4}	1.7×10^{-4}	$< 0.02 \times 10^{-4}$
Cholesterol	1.25	3.07×10^{-4}	$< 0.02 \times 10^{-4}$ *	$< 0.02 \times 10^{-4}$
Oleic acid	1.25	3.07×10^{-4}	$< 0.02 \times 10^{-4}$	$< 0.02 \times 10^{-4}$
Triolein	1.25	3.07×10^{-4}	$< 0.02 \times 10^{-4}$	$< 0.02 \times 10^{-4}$

The procedure is described in the text. * Limit of sensitivity: $0.02 \times 10^{-4} \mu\text{mol}$.

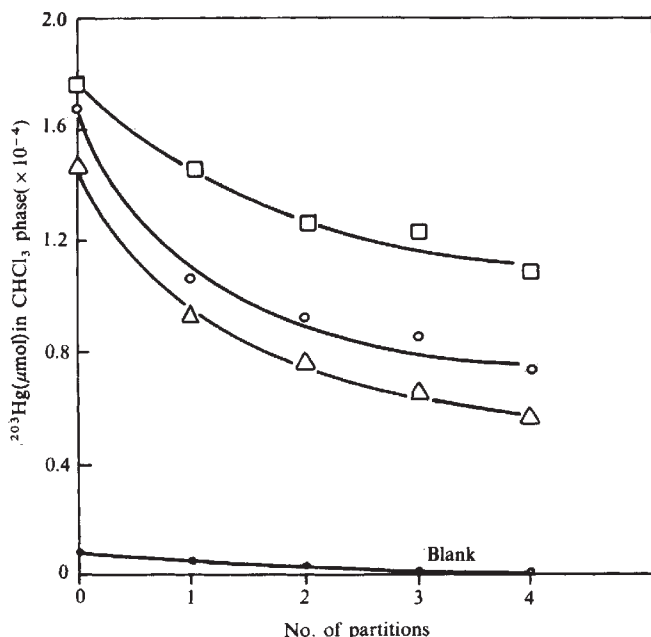


Fig. 1 The release of mercuric ions by phospholipid after partitioning with buffer. Procedure is given in text. □, Phosphatidyl serine; ○, phosphatidyl ethanolamine; △, phosphatidyl choline.

that considerable amounts of the bound $^{203}\text{Hg}^{2+}$ and $\text{CH}_3^{203}\text{Hg}^+$ were freely dissociated from the metallolipoproteins on removal of unbound mercurials suggests that the reactions between the mercurials and sites on the proteins may have been partly responsible for the weak bonds formed between the mercurials and the lipoproteins. Nevertheless, the major differences in the uptake and release of the ^{203}Hg between the lipoproteins and the apoproteins (Table 1) imply that lipids also played an important role in the interactions.

The studies with the $\text{CHCl}_3:\text{H}_2\text{O}$ model system showed that PC, PE and PS readily bind and release Hg^{2+} (Table 2, Fig. 1). Thus, phospholipids form readily dissociable complexes with Hg^{2+} in CHCl_3 solutions. The active incorporation of $^{203}\text{Hg}^{2+}$ by PC, PE and PS suggests that phospholipids may play an important role in binding this ion in the lipoproteins. Moreover, phospholipids may well release the bound Hg as the Hg^{2+} concentration of the surrounding medium is decreased.

No evidence was found to indicate that neutral lipids or fatty acids participate directly in the binding of Hg^{2+} ; however, the involvement of these lipids in sequestering Hg^{2+} in the lipoproteins cannot be ruled out.

The data did not indicate that CH_3HgCl binds with either the phospholipids, neutral lipids or fatty acids examined (Table 2). Thus, the interactions of methylmercury with the lipid portions of the lipoproteins (Table 1) may be related

primarily to the high lipid solubility of this molecule¹⁸. In fact, considering the amounts of methylmercury incorporated into the lipoproteins and apoproteins, it seems that the high lipid content of the VLDL and LDL (Table 1) may account for the greater incorporation of $\text{CH}_3^{203}\text{Hg}$ into these lipoproteins relative to their apoproteins in comparison to HDL and apo HDL. It was suggested previously that the greater toxicity of methylmercury in comparison to inorganic mercury is due to the greater lipid solubility of the alkylated derivative¹.

The present initial studies implicating the serum lipoproteins in sequestering mercurials raises important questions about the significance of such interactions. Do mercurials disrupt the surface charge distributions or alter other structural features of the lipoproteins so that their physiological or immunological properties¹⁹ are impaired. Detailed studies directed toward the possible role of the serum lipoproteins in the transport of the mercurials seems appropriate. Further, it would be interesting to know whether the observed interactions of the serum lipoproteins with mercuric and methylmercuric ions are intimately related to the toxic effects of these mercurials on vital organs, such as the brain.

WILLIAM L. REICHERT
DONALD C. MALINS

Environmental Conservation Division,
Northwest Fisheries Center,
National Oceanic and Atmospheric Administration,
Seattle, Washington 98112

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Is the Slow Worm a Batesian Mimic?

THE phenomenon of Batesian mimicry, where an edible animal gains an advantage by mimicking a distasteful or dangerous animal showing a warning pattern, is well known and has been described by many authors¹⁻⁴. The advantage gained by a mimic decreases if the mimetic form becomes too common, since a predator may learn that the warning signal mimicked is a sign of edibility rather than danger; thus selection for the mimic is density-dependent which, as shown by Haldane and Jayakar⁵, will often lead to a balanced polymorphism where both mimetic and non-mimetic forms are retained in the population. Matessi and Cori⁶ suggest that mimetic inheritance is often sex-limited so that the expression of mimicry is limited to one sex (usually the female according to Sheppard³) although individuals of the other sex may have the mimetic genotype. It is suggested here that the slow worm (*Anguis fragilis* L.) has a form of colouration exhibited by the young and some adult females which is a mimic of the warning pattern of the adder (*Vipera berus* L.).

Although adders are very variable in their ground colour and markings, one characteristic that immediately distinguishes them from the other British snakes is the dark, zigzag line down the centre of the back. The pattern may be broken up or more like a continuous stripe, but it is still the most distinctive feature of the snake and almost certainly acts as a warning to potential enemies (although the pattern may act as camouflage in dead bracken (E. P. Killips, personal communication)). Certainly, both Smith⁷ and Prestt⁸ say that the chief enemy of the adder is man and, although adders are sometimes taken by the buzzard (*Buteo buteo* L.) and the hedgehog (*Erinaceus europaeus* L.), they seem to have few natural enemies. Prestt⁸ found that even 3-d-old adders could inflict a poisonous bite but that generally when domestic animals were bitten they were not killed, suggesting that animals could learn, by experience, to avoid adders without the lessons proving fatal. Reid⁹ confirms that, as well as a prey-killing bite, venomous snakes generally have a defence or warning bite which injects little or no venom but allows the snake to escape.

Slow worms do not usually grow as large as adders and have upper parts which vary considerably in ground colour, a variation probably associated with their habitat. All new-born slow worms have a thin black zigzag or stripe down the middle of the back but, as the animals reach sexual maturity, all males and some females lose the stripe. It seems likely that the females which retain the stripe are mimicking the adder, and the presence of the dimorphism in the females but not the males supports this hypothesis. The question that follows is, when will the mimicry be of any advantage?

The slow worm is not often seen in daylight, preferring to hide under stones, logs or leaves or even burrowing in light soil. It does appear in the day, however, in early spring when mating, and in the late summer when pregnant females bask in the sun. Both the adder and the slow worm are ovoviviparous, an uncommon feature among the Squamata, so the eggs are less likely to fall victim to predators and the mother can warm the eggs inside her by basking, or shelter them from extremes of heat and cold by taking cover. The mother and young are probably most vulnerable near the time of birth, so if the female adder gains some protection from its aposematic colouration at this time, the female slow worm and her young might also be protected by mimicking the warning. The loss of the distinctive pattern by all the males and some of the female slow worms shows the dynamic balance that must exist between selective advantage and frequency of the mimetic form.

If the maximum advantage is to be gained from the mimicry, the active periods of mating and birth in the slow worm should correspond with those of the adder. Figure 1

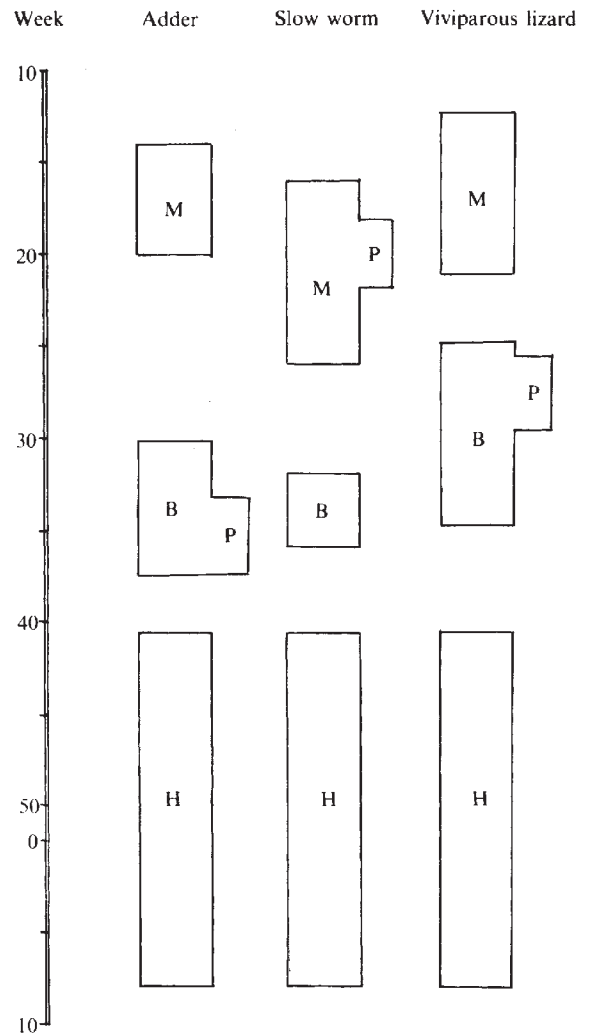


Fig. 1 Activities throughout the year of the adder, slow worm and viviparous lizard. H, Hibernation; M, mating; B, birth; P, peak period.

shows the activities through the year of the adder, the slow worm and viviparous lizard (*Lacerta vivipara* Jacquin) adapted from Smith⁷. Mating in the slow worm tends to occur towards the end of the adder's mating period but birth takes place over a very short period of time corresponding to the peak birth period of the adder. By way of comparison, the viviparous lizard gives birth to its young very much earlier, suggesting that the slow worm has evolved such that the mimicry gives maximum advantage at the most important time.

The hypothesis that the slow worm is a Batesian mimic of the adder is difficult to test experimentally, although a laboratory experiment using adders, slow worms and assorted predators could be devised. The evidence presented in Fig. 1, however, supports the hypothesis, and it is hoped to estimate the selective value of the mimetic form by mark recapture as described by Manly¹⁰. Data are currently being collected to discover whether the frequency of the patterned form is related to the frequency of adders, and of particular interest are areas where slow worms but not adders are found, such as Radnor, South Lincolnshire, the Outer Hebrides and the Channel Islands. Islands should be particularly informative since the effects of natural selection in the absence of migration can be seen there. N. Jee and R. Brehaut report that they have never observed an adult slow worm with a dark stripe on Guernsey, but they have not been looking out for the pattern so it could have escaped

their notice; however, their evidence supports the hypothesis suggested here.

ROBERT H. SMITH

Department of Zoology,
University of Reading,
Reading, Berkshire, RG6 2AJ

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Differential Climatic Selection in Natural Population of Land Snail *Cepaea nemoralis*

DURING a study of the energetics of a population of the polymorphic land snail *Cepaea nemoralis* L. on the sand dunes at Braunton Burrows, North Devon, numbers of dead and dying snails were collected during the daylight hours in the summer. They showed similar symptoms, being unable to retract the foot which had become dried, discoloured and unresponsive to touch stimuli. Some animals were unable to retract the optic tentacles and in some, the genitalia protruded. Snails found in this condition, although alive (as evidenced by sensitivity of the mantle area) early in the day, were usually dead by evening.

At first a parasitoid fly was suspected as the cause of death, but on incubating the dead animals, only two flies emerged: a specimen of the calliphorid *Sarcophaga nigri-ventris* and a muscid (*Fannia* sp.). Both are opportunist scavengers¹, but *Sarcophaga* has been found parasitising snails. Later experiments on the upper lethal temperature of snails produced symptoms identical to those found in the field and it was assumed that the animals had died as a result of high temperatures.

The animals were collected on two occasions in 1970 and six in 1971 from an area of known size (1,600 m²). The total number collected and the number of deaths per week are shown in Table 1.

Table 1 Heat deaths in an area of 1,600 m²

	Total dead snails collected	Deaths (week ⁻¹)
1970 1 Oct.	206	—
15 Oct.	30	15.0
29 Oct.	9	4.5
Total	245	Average 9.75
1971 29 April	25	5.0
13 May	16	8.0
24 June	15	2.5
22 July	10	2.5
29 July	4	4.0
16 Aug.	6	1.5
Total	76	Average 3.92

The mean population of adult snails in the area was 1,900 and the mean weekly survival rate in a sub-area used for a capture-mark-recapture study was about 0.9987, which com-

pares with 0.9566 calculated by the Jolly² capture-mark-recapture census over the same area.

There is no clear seasonal trend in the mortality, but it has been shown³ that almost any sunny day from March to September can produce temperatures at the snails' upper lethal limit near the grass surface. For short periods of exposure (0.5 h) the upper lethal temperature is about 43° C.

The frequencies of colour and banding morphs of the dead animals were compared with those prevailing in the population, based on live animals collected during random quadrat sampling. In this instance, a collection made from another area of the sand dunes (Site 2), with rather different morph frequencies is included. Table 2 shows the morph frequencies of the dead snails with the expected frequencies in brackets.

Table 2 Morph analysis of dead shells collected during 1970–1971

	Yellow	Pink	Brown	Total
1970 Site 1				
00000	0 (0.5)	3 (10.5)	12 (11.3)	15 (22.3)
00300	12 (12.7)	78 (81.1)		90 (93.8)
12345	10 (17.6)	130 (111.2)		140 (128.8)
Total	22 (30.8)	211 (202.8)	12 (11.3)	245
1971 Site 1				
00000	0 (0.2)	0 (3.3)	1 (3.5)	1 (7.0)
00300	4 (4.0)	23 (25.2)		27 (29.2)
12345	4 (5.5)	44 (34.5)		48 (40.0)
Total	8 (9.7)	67 (63.0)	1 (3.5)	76
1970 Site 2				
00000	5 (15.2)	27 (45.6)	1 (0.0)	33 (60.8)
00300	10 (21.3)	291 (258.1)		301 (279.4)
12345	4 (21.3)	169 (145.5)		173 (166.8)
Total	19 (57.8)	487 (449.2)	1 (0.0)	507

The differences between the observed and expected frequencies may be tested by a log likelihood ratio test⁴; the *G* statistic degrees of freedom and probabilities are shown in Table 3.

Table 3 Comparison of observed dead shell morph frequencies

	<i>G</i>	d.f.	<i>P</i>
1970 Site 1	15.48	5	0.01–0.005
1971 Site 1	6.76	3	0.1–0.05
1970 Site 2	52.49	5	<0.005

In all cases, differences between the observed and expected frequencies are apparent and are highly significant in the cases of Sites 1 and 2 in 1970. These two collections were much larger than the 1971 one and included shells which had been dead for some time.

The trend in all three collections is similar: lower numbers of unbanded and yellow shells than expected and correspondingly higher numbers of banded and pink shells, suggesting that yellow and unbanded animals are more resistant to high temperature stress than pink and banded animals. Schnetter⁵ suggested that physiological selection favoured unbanded yellow shells of *C. hortensis* in dry sunny situations, and in laboratory experiments, Boettger⁶ (in *C. hortensis*) and Lamotte⁷ found yellow and unbanded animals to be more resistant to high temperatures. Yellow-shelled animals selected a higher temperature in a temperature gradient than pink-shelled ones in a study by Sedlmair⁸.

It is also interesting to note that laboratory experiments on the albedo of empty shells⁹ showed that yellow unbanded shells reached a temperature of 1 to 2° C lower than that attained by pink banded shells (especially where the bands were fused producing an effectively black shell) under the same radiation conditions. This small difference may be important in producing differential survival when temperatures are near the upper lethal temperature.

The polymorphism of sand-dune populations of *C. nemoralis* has been discussed by Cain⁹. He suggests that the overall distribution of morph frequencies is consistent with the action of visual selection by predators. On the present site, thrush predation occurred only in the winter months on buried, hibernating snails, and thus with no opportunity for visual selection. Climatic factors are indicated by Cain to be of some importance, notably the relationship between high frequencies of the dark brown morph and areas where cold air accumulates at night, but also the higher frequency of snails with yellow mid-banded shells on sunny south-west facing slopes as opposed to higher frequencies of snails with pink five-banded shells on north and east facing slopes. My findings provide what seems to be the first direct evidence¹⁰ of differential climatic selection in the field and support Cain's conclusion that climatic selection may over-ride visual selection in the maritime duneland situation.

ALASTAIR M. M. RICHARDSON

Department of Zoology,
University of Tasmania,
Box 252C, G.P.O.,
Hobart,
Tasmania, 7001

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Habitat selection in titmice

HABITAT selection has important ecological and evolutionary consequences for birds as for other animals¹⁻⁴, yet although there is abundant evidence from natural observation that it occurs, little is known of the cues by which habitats are recognised, nor the role of experience in determining such preferences⁵⁻¹⁰.

In Europe the coal tit (*Parus ater*) is primarily conifer-dwelling whereas the closely related blue tit (*Parus caeruleus*) lives mostly in deciduous woods¹¹. There is some overlap, and where this occurs there are differences in habitat utilisation¹²⁻¹⁴. The experiments described here were designed to show if hand-reared birds with no experience of vegetation would show any preference for the type of vegetation in which they are usually found. This type of experiment has been conducted with rodents¹⁰, and with birds¹⁵, but with the latter the results were not clear, so that the question of whether 'naive' birds can make this distinction remains.

The experiments were carried out with a mixed group of naive birds, eight blue tits and six coal tits. They were taken from the nest 10 d after hatching and raised together until the tests. At 8 weeks the birds were presented with a composite branch hung from the ceiling of the aviary. The branch was made from two smaller ones lashed together side-by-side and each about 7 feet long. One was of Oak (*Quercus robur*) and the other of Scots Pine (*Pinus sylvestris*). The birds showed immediate interest in the branches and searched them assiduously for about an hour after their

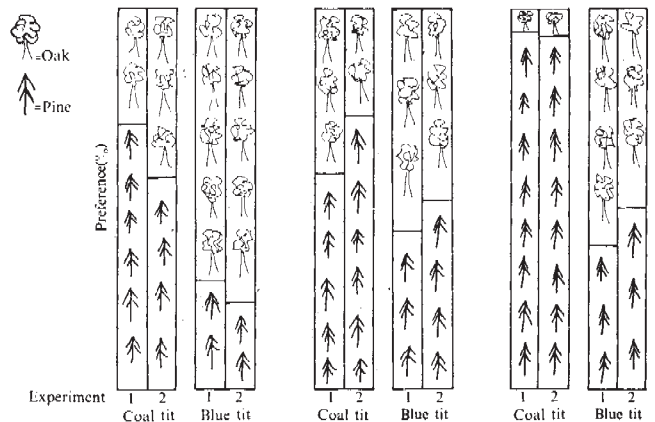


FIG. 1 Percentage preference for oak and pine by the coal tit and blue tit in the three pairs of experiments. a, with complete branches; b, branches stripped of leaves and, c, leaves alone.

introduction; the branches on which the birds were perched were recorded every minute. The experiment was conducted twice, a few days apart. The results (experiment a, Table 1 and Fig. 1) show that there is a significant difference in preference, each species preferring that type of tree in which it is most often found in the wild.

This method has several advantages: because the twigs of the two branches are closely interwoven, one can be sure that differences in preference cannot reflect differences in the favoured part of room, for instance with reference to shade or temperature. Nor can it have been produced by any tendency to flock with or avoid members of their own or the different species, as a bird attracted to flock with or avoid another would have had both species of branch within a few inches. Imitation¹⁶ is unlikely to have occurred because the birds were frequently out of sight of one another on the branch, and when feeding they seemed to observe the other birds very little. As identical branches were presented for both species of birds, differences in the amount of twig available for perching, or food available on the branches could not have produced the results.

In previous years experiments conducted with individual birds gave variable results because the branches die quickly, and therefore different pairs of branches had to be used for

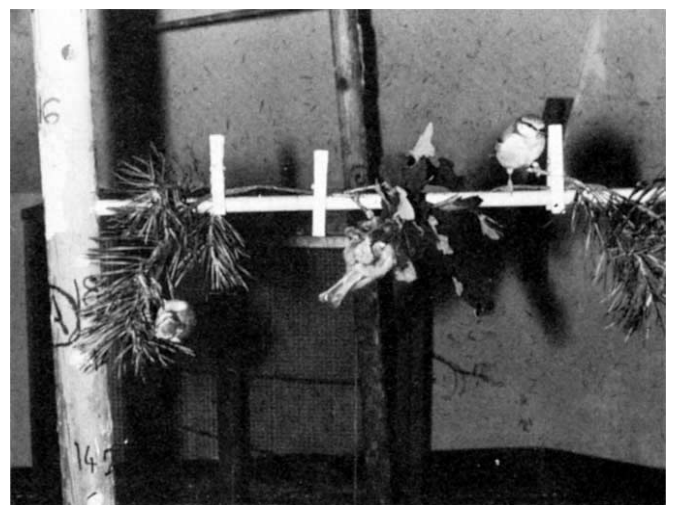


FIG. 2 Arrangement of leaves alone in experiment c.

TABLE 1 Table of bird-records for coal tits and blue tits perched in oak and Scots pine in the three pairs of experiments.

		Oak	Pine	% Pine	Oak	Pine	% Pine	Oak	Pine	% Pine
Experiment 1	Coal tit	27	51	65	63	85	57	1	16	94
	Blue tit	106	44	29	116	83	42	42	26	38
Experiment 2	Coal tit	67	p*	56	21	p†	72	2	p*	93
	Blue tit	159	48	23	61	62	50	44	40	48
			p*			p†			p*	
		Complete branches (a)			Branches stripped of leaves (b). Leaves alone (c)					

* Significance level $<0.1\%$ on $2 \times 2 \chi^2$ test.

† Significance level $<1\%$ on $2 \times 2 \chi^2$ test.

each bird. It was noted that all individuals of each species seemed to show the same preference although it is possible that there were quantitative differences. The intense activity of the birds while searching meant that when successive observations were made on the same individual it was likely to be in a different region of the branch. There was no trend of increasing or decreasing preference with time for either species.

To examine the effect of leaves on the preference, two more experiments (b and c) were undertaken. In one the birds were presented with a composite branch stripped of leaves, in the other with leaves in a manner shown in Fig. 2. The leaves were pegged in bunches onto wooden perches. The results (Table 1) show that there is a significant difference in preference both for the bare branches and for the leaves of the tree in which they are usually found. Figure 1 illustrates that in the last experiment the blue tits distributed their visits more evenly between the two branch types than the coal tit who landed very little in oak leaves.

These experiments show that these two bird species can actively select their usual habitat in the absence of any previous experience of it. To what extent this preference may be modified by subsequent experience remains to be seen.

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LINDA PARTRIDGE

Department of Experimental Psychology,
South Parks Road,
Oxford

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Coral Growth Related to Resuspension of Bottom Sediments

THE determination of coral skeletal growth, from both a biological and a geological standpoint, has long presented difficulties in ease and accuracy of measurement. As a consequence, factors which limit growth are only beginning to be investigated. Radiometric methods using natural radioactive series nuclides have recently been used to determine the growth rate of certain corals^{1,2}. In addition, X radiographs coupled with ⁹⁰Sr-induced autoradiographs have demonstrated that at least some hermatypic corals record annual growth bands in their skeletons^{3,4}. Once annual banding is confirmed, measurement of band widths allows precise determination of yearly growth increments. It is thus possible to compare inter- and intraspecific coral growth rates from different areas with pertinent environmental variables and to deduce possible correlations. We describe here an investigation of the effects of resuspension of bottom sediments on the growth rate, as determined by a ²²⁸Ra technique and X radiography of *Montastrea annularis* in different parts of Discovery Bay, Jamaica. Such a study is unique both because the annual nature of growth bands is established for the most important reef-forming coral in the Caribbean⁵ and because radiometric and radiographic techniques are extended to measure growth rates as a function of environmental parameters.

The effect of sedimentation on coral growth has never been extensively analysed. Marshall and Orr⁶ demonstrated that although relatively high rates of sediment fouling will kill coral species, most corals can withstand a low sediment input by active physical removal of the sediments. Yonge⁷ states from findings in Professor T. Vaughan's notes that sand was removed, although slowly, from the surface of *M. annularis* with much mucus being extruded, and has discussed also a coral species' ability to clear itself of sand by distension of the coenosarc with water⁸. More recently, the self-cleaning ability of certain corals has been further discussed⁹⁻¹¹. Constant energy expenditure by corals for removal of sediment particles might be expected to decrease growth rate. In addition, hermatypic corals are known to be dependent on light for skeletal growth¹², so that the decrease in available light intensity due to turbidity might also have a marked effect on growth rate.

After preliminary observations of the shallow rear zone and adjacent soft bottom fauna of the lagoon of Discovery Bay, we measured resuspension rates of bottom sediments in order to assess bottom stability and turbidity¹³. Three stations were established (Fig. 1) on the soft carbonate sediment bottom near the rear zone of the reef. Resuspension was measured in duplicate at each of the three stations by placing sediment traps 50 cm above the bottom and weighing the collected sediment. Weather conditions were identical throughout the experiment (3/16/73-3/24/73 for stations A and B, 3/17/73-3/25/73 for station C), and no significant variations in either salinity or temperature were observed between the stations

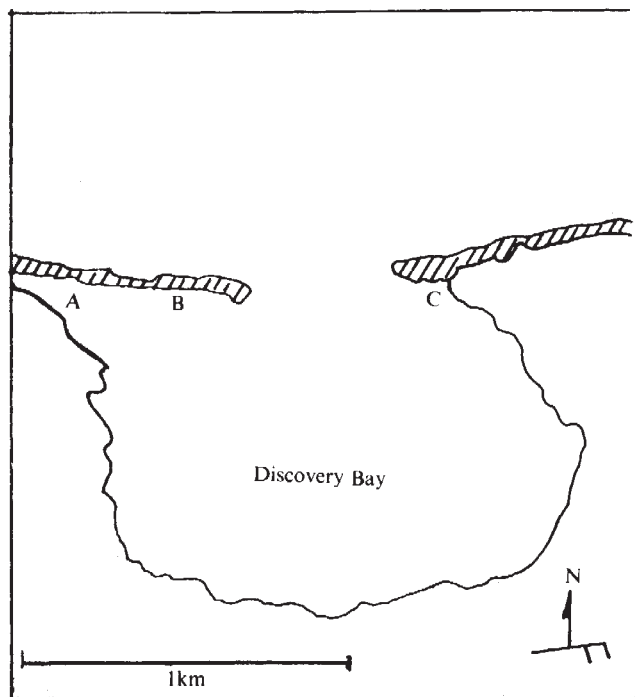


Fig. 1 Sketch map of Discovery Bay, Jamaica, showing station locations (A-C). Stippled area represents reef.

(R. C. A. and R. E. D., in preparation). The resuspension rates determined were thus an integrated measure of bottom instability under the following diurnal conditions: throughout the morning hours the lagoon is calm and clear, but by afternoon the Northwest Trades, which blow daily, have driven water turbidity to a maximum, and by evening the lagoon is again calm and clear and remains that way all night. Jackson¹⁴

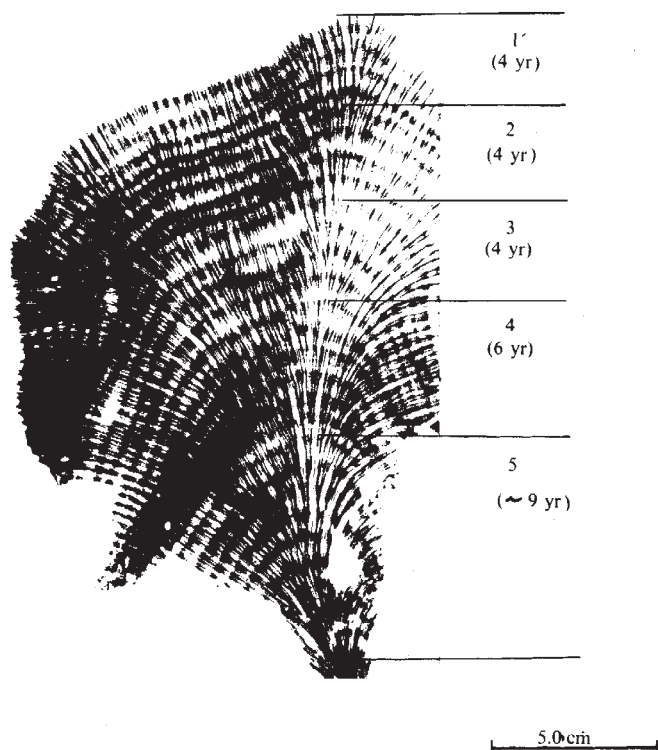


Fig. 2 X-radiograph positive of a specimen (A2) of *Montastrea annularis* from station A showing annual bands and locations of five samples for ²²⁸Th analysis.

presents a number of environmental variables measured at the eastern part of Discovery Bay over the period of a year. His data indicate that March is characterised by turbidity values relatively lower than those for winter and slightly higher than those for summer. Our data suggest that a gradient of increasing resuspension from east to west exists in the Bay.

Coral heads of various species were collected from the rear zone of the lagoon at depths of 2–4 m, within 50 m of the station locations. *M. annularis* was chosen for intraspecific analysis because it is a representative species and also the major Caribbean reef framework coral⁵ and has been shown previously to exhibit skeletal banding³.

Specimens collected were hemispherical to semi-hemispherical, not larger than 50 cm in height, and taken only from areas of living reef. The corals were first sectioned into slabs about 2 cm thick on a rock saw. These slabs were oriented so as to include the point of highest relief on the actively growing coral surface and also the midpoint of the base. The slabs were prepared and X radiographed using the techniques of Knutson *et al.*³ except that slab thicknesses of 0.3–0.5 cm were used. Band widths of each specimen were measured along several line transects drawn on the X-ray positive (Fig. 2), one transect always drawn so as to include the axis of maximum growth. Because of the imperfect hemispherical shape of specimens, the other transects were drawn for calculation of an 'average' estimate of upward growth. Table 1 gives the results of these measurements. Values for coral growth rate are weighted means derived from calculation of the means and standard deviations of each transect in a slab. The maximum growth rate given for each station is a weighted mean of transects along the axes of maximum growth of all corals at that station, and the 'average' growth rate of a station is a weighted mean of all transects. Weighted means and associated weighted standard deviations have been used to favour individuals that show a more even growth. This seems reasonable in a biological sense because we lack information on the history of each coral.

Table 1 Coral Growth Values for Stations and Individual Heads Collected at Each Station

Corals and stations	Number of transects	Average year/transect	Growth rate (cm yr ⁻¹) weighted mean $\pm$ weighted σ
Station A			
A1	2	16	0.50 $\pm$ 0.059
A2	2	21	0.60 $\pm$ 0.082
A3	2	6	0.72 $\pm$ 0.055
Maximum (contains axis of maximum growth transects only)			0.65 $\pm$ 0.050
Average (contains all transects for station)			0.62 $\pm$ 0.036
Station B			
B2	3	6	0.82 $\pm$ 0.055
B4	3	6	0.83 $\pm$ 0.077
Maximum (contains axis of maximum growth transects only)			0.90 $\pm$ 0.063
Average (contains all transects for station)			0.82 $\pm$ 0.047
Station C			
C2	3	4	1.06 $\pm$ 0.085
C3	2	12	0.58 $\pm$ 0.108
C7	3	10	0.88 $\pm$ 0.075
Maximum contains axis of maximum growth transects only			1.00 $\pm$ 0.082
Average (contains all transects for station)			0.88 $\pm$ 0.050

The determination of the rate of growth of *M. annularis* was done by α spectrometric analysis for *in situ* ²²⁸Th growing in from ²²⁸Ra which had been precipitated from seawater with the aragonitic coral skeleton. The chemical procedure was similar to that of Moore *et al.*². Samples including sufficient growth bands to yield about 50 g were obtained for a specimen from station A by comparison of a slab with its associated X radiograph (Fig. 2).

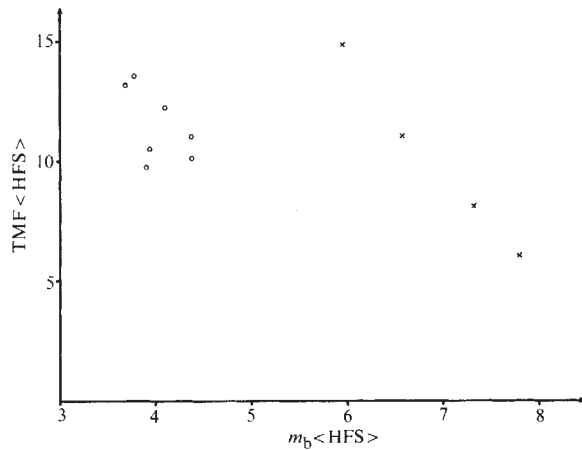


Fig. 3 ^{228}Th specific activities obtained for skeletal intervals shown in Fig. 2. The curve is the predicted ^{228}Th ingrowth for an initial ^{228}Ra value of 4.0 d.p.m. per 100 g coral.

The *in situ* ^{228}Th method depends on the assumption that the coral incorporates ^{228}Ra into its skeleton at a fixed ratio to Ca from the surrounding seawater, and that this initial ratio has been constant throughout the life of the coral. Analysis for ^{228}Th at time intervals defined by the growth bands should then conform to an ingrowth curve of ^{228}Th towards transient equilibrium with the initial ^{228}Ra specific activity. In Fig. 3 the 1 sigma counting uncertainties on the ^{228}Th analyses are entered at the midpoint of the time spans except for sample 5 which has been weighted towards younger values because of volume considerations in the triangular sample. The curve in Fig. 3 corresponds to the predicted ^{228}Th ingrowth for an initial ^{228}Ra specific activity of 4.0 d.p.m. per 100 g. Recent measurements of ^{228}Ra in Caribbean surface water¹⁵ lie in the range 3.7 to 4.4 d.p.m. per 100 kg, so that *M. annularis* seems to exhibit little discrimination against Ra relative to Ca. However, a specimen of *Stephanocoenia* sp. taken at a depth of 25 m in Discovery Bay is reported¹ to have an initial value of 1.3 d.p.m. ^{228}Ra per 100 g. The reason for this difference is not known.

Resuspension values are plotted against both 'average' and maximum coral growth rates in Fig. 4. There is a definite inverse trend between resuspension values and coral growth in both cases with increased resuspension and lowered growth rates from east to west. The stress gradient thus defined results from both physical and biological factors. The North-east Trades couple with bottom stability factors such as benthic infaunal burrowing activities and sediment-binding algae (R. C. A. and R. E. D., in preparation). The standard deviation of both maximum and 'average' growth also decreases as resuspension increases, indicating that not only does high resuspension hinder coral growth, but also decreases the variability in growth. This suggests that the ability of the coral to respond to other favourable, or at least less limiting, environmental variables is reduced or overshadowed by high resuspension.

This study demonstrates the importance of sampling location in any study of coral growth rate. In selected areas in Florida and the Caribbean various workers (refs 3, 16-18, and I. G. MacIntyre and S. V. Smith, written communication from paper presented at 2nd Int. Symp. on Coral Reefs, Great Barrier Reef (1973)) have found a range of 0.50-1.7 cm yr^{-1} for skeletal growth of *M. annularis*. If all the rates are intercomparable, it is obvious that one species can have values ranging over a factor of three without gross morphological indications. Further, our data indicate that a factor limiting coral growth is closely associated with the intensity of resuspension of bottom sediments. Other factors, such as temperature, light availability,

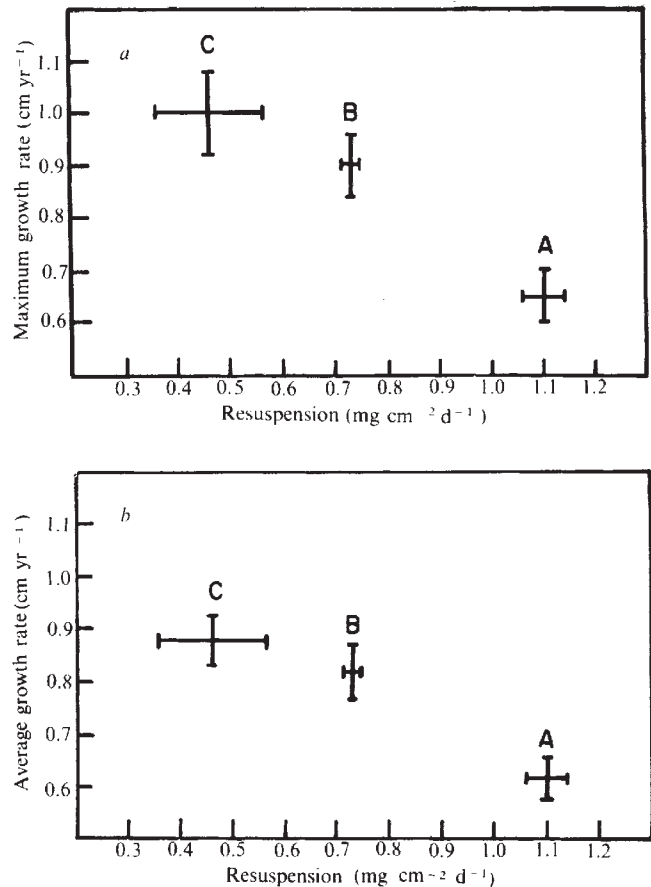


Fig. 4 a, Maximum and, b, average, upward coral growth (error bars = 2σ) against ranges of resuspension values for each station.

salinity and nutrient supply, are clearly also important. An extensive analysis on a reefwide scale would be very useful, not only in delineating average growth conditions but also in uncovering other possible limitations on coral growth. When these factors are understood, the effects of perturbation, whether by man or nature, can be assessed. Then problems such as the rate of recovery following growth disruption or retrieval of historical information based on 'dendrochronological' analysis can be approached.

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RICHARD E. DODGE
ROBERT C. ALLER
JOHN THOMSON

Department of Geology and Geophysics,
Yale University,
New Haven, Connecticut 06520

Received August 28; revised October 26, 1973.

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that at least aspects of the acute phase of the syndrome may be remedied.

A. M. HARTHOORN

Nature Conservation Division, P. Bag X209,
Pretoria

K. VAN DER WALT

Faculty of Veterinary Science,
Onderstepoort

E. YOUNG

Division Veterinary Services,
Kruger National Park,
P.O. Skukuza,
South Africa

Received July 8; revised September 28, 1973.

Possible Therapy for Capture Myopathy in Captured Wild Animals

CAPTURE myopathy (so-called overstraining disease) in wild animals has gained increasing prominence over the past decade as attempts to capture remaining nuclei of rare species for relocation and restocking, are rendered abortive by high death rates. The proportion of deaths is usually highest in the calves and in gravid females. High death rates in diminishing animal species such as tsessebe (*Damaliscus lunatus*) have occurred in spite of all precautions, such as the use of a helicopter to reduce the time between alerting the subject animal and the placement of a syringe containing suitable immobilising compounds.

Investigation on a number of species including blesbok (*Damaliscus dorcas*) and wildebeest (*Connochaetes taurinus*), has shown that an acute metabolic acidosis seems to be the causative factor in several at least of the manifestations of capture myopathy and death. A fall in the blood pH and attendant factors, namely base excess, buffer base, plasma and total bicarbonate, occur most precipitously in those animals pursued intensively for short distances of 1 to 2 km. The deaths, with symptoms of capture myopathy seem to be due mainly at least to acute and profound acidosis rather than, as was widely believed, to exhaustion and overstraining of muscles and vital organs.

The fall in blood pH continues after the animals have been captured. The crating of captive animals such as zebra (*Equus burchelli*) in itself causes acidosis, so that little or no abatement of the condition may be expected during subsequent transport.

We have treated the syndrome induced experimentally in nine wild zebra (weighing 200–250 kg) pursued over short distances. We infused them intravenously with 1,000 mEq of sodium bicarbonate dissolved in Normosol solution. Normosol solution (Abbott, Johannesburg) contains the following ions: Na⁺, 140; K⁺, 5; Mg²⁺, 3; Cl⁻, 98; acetate, 27; gluconate, 23 mEq l⁻¹. Approximate pH 7.4. This therapy was successful. Six animals were treated and all survived. The treatment, given over 5 to 10 min, was carried out 30 min after capture and resulted in immediate and spectacular amelioration of the predominant clinical symptoms such as dyspnoea and cardiac dysfunction: heart and respiratory rates were slowed, and the heart sounds became clearly audible and defined. Skeletal muscular spasm, as evident in untreated animals was prevented, and there was an immediate improvement in the animals' reactions and general condition.

The three untreated animals died within 12 h apparently of pulmonary oedema with *post mortem* findings exhibiting characteristic lesions of capture myopathy.

We do not wish to oversimplify the problem presented by capture myopathy in conservation measures, nor exclude genetic or acquired predisposition. It does appear, however,

Ribonuclease activity of wheat leaves and rust infection

REMARKABLE changes in the levels and properties of ribonuclease (RNase) occur following the inoculation of a susceptible variety of flax (*Linum usitatissimum* L., var. Bison) with flax rust uredospores (*Melampsora lini* (Ehrenb.) Lev, race 3)¹. The increase in RNase activity occurs in two distinct phases; an early phase between 2 to 4 d after inoculation (early RNase) and a later phase, beginning 5 d after inoculation (late RNase). These increases in RNase activity are accompanied by substantial changes in the properties of the enzyme including thermal stability, diethylpyrocarbonate-sensitivity and substrate preference¹. Further studies (A. K. C., M. S., and L. A. S., unpublished) have revealed that these changes in the properties of RNase are due, at least in part, to the formation of new RNase molecules. These have kinetic and catalytic properties that are quite distinct from both of those 'host' RNase from healthy flax cotyledons and 'rust' RNase from flax rust uredospores or from rust mycelium grown axenically in a chemically defined medium. That changes in late RNase activity are specifically elicited by compatible host-parasite interactions is indicated by the appearance of late RNase which is not detectable in a resistant variety of flax (Bombay) inoculated with race 3 of *Melampsora lini*¹. Furthermore, the characteristic bimodal increase in RNase activity and the accompanying changes in various properties of the enzyme have been found in a number of other rust-infected plants including pine and *Ribes* (A. E. Harvey, A. K. C., M. S. and L. A. S., unpublished), and mechanical injury of the healthy host tissues causes a significant increase in the level of RNase. The enzyme remains strictly 'host-type', however, no change in its physical or catalytic properties being detectable (ref. 1 and A. E. Harvey, A. K. C., M. S., and L. A. S., unpublished). Here we describe the effect of inoculation with uredospores of three races (56, 56A and Australian race 126 ANZ-6, 7) of wheat stem rust (*Puccinia graminis* (Pers.) f. sp. *tritici* Eriks. and E. Henn.) in causing quantitative and qualitative changes in the RNase activity of paired, near-isogenic lines of wheat (*Triticum aestivum* L.) derived from the variety Chinese Spring and carrying the alleles *Sr6* and *sr6* (refs 2 and 3), respectively. Both lines give susceptible reactions (infection type 4 (ref. 4)) with race 56A, independent of temperature. The homozygous recessive, *sr6*, imparts susceptibility (infection type 4) to races 56 and 126 ANZ-6, 7. The dominant allele, *Sr6*, confers a temperature sensitive reaction to these races; resistance is expressed (infection type 0 (ref. 4)) at 19 to 21° C but breaks down (infection type 4) at higher temperatures (25 to 26° C). A few intercellular hyphae are produced at each infection site at 19 to 21° C. These survive for several days and resume growth when infected plants are transferred to 25 to 26° C; but, in our experience, the longer infected

plants are held at 19 to 21° C before transfer, the smaller are the number and size of the sporulating pustules to be developed.

The isolate of the Australian race, 126 ANZ-6, 7, was provided several years ago by Dr P. G. Williams and has been maintained by us. It was included in this study because it is readily grown in axenic culture^{5,6} and is therefore a convenient source of mycelial RNase. The results indicate a positive correlation between increased late RNase activity and the ability of the host to support the growth of the pathogen, and for qualitative changes in the properties of both early and late RNase.

Wheat seedlings were grown in a growth chamber at 20° C with 16 h illumination at 800 to 1000 foot-candles and 8 h darkness daily. Fully-expanded primary leaves were inoculated by dusting with a mixture of uredospores and talc or talc alone (controls). After dusting, the plants were placed in a humidity chamber¹ for 12 h and then returned to the growth chamber. Batches of healthy and inoculated plants were transferred at intervals to a second growth chamber maintained at a daylight temperature of 26° C. The number of sporulating pustules (infection type 3-4) was recorded 1 or 3 d after they first appeared. RNase was extracted from excised primary leaves and partially

purified by acidification at pH 5.0 as described before¹.

Figure 1 shows the RNase specific activity ratios (inoculated: healthy) of the two lines of wheat at different times after inoculation and incubation at 20° C and 26° C. The specific activity of the RNase from healthy plants varies only slightly during the experimental period, so that an increase in the ratio is the result of an increase in the specific activity of RNase in the inoculated leaves. The results in Fig. 1 indicate that the characteristic bimodal increase in RNase activity occurs only in host-parasite combinations that result in susceptible (type 3 to 4) reactions (*Sr6*-race 56 and *Sr6*-race 56A at 20° C; *Sr6*-race 56 and *Sr6*-race 126 ANZ-6, 7 at 26° C). In combinations that result in resistant (type 0 or 0₁) reactions (*Sr6*-race 56 and *Sr6*-race 126 ANZ-6, 7 at 20° C) only the early phase of RNase increase occurs. These results show a positive correlation between changes in the levels of RNase and the continued growth of the rust in host tissue.

Figure 2 shows the development of late RNase in groups of *Sr6* plants transferred from 20 to 26° C at various times after inoculation with race 56. Plants transferred 12 h (Fig. 2a) after inoculation show the normal pattern of early and late RNase activity exhibited by plants inoculated and maintained at 26° C (Fig. 1c). In plants maintained at 20° C for 24 h (Fig. 2b), 5 d (Fig. 2c), and 13 d (Fig. 2d) before transfer to 26° C, the appearance of late RNase activity is increasingly delayed; in plants maintained continuously at 20° C, no late RNase activity develops (Fig. 2e and Fig. 1a). In addition, the initial appearance of sporulating rust pustules (indicated by inverted arrows in Fig. 2) is increasingly delayed and their number is progressively reduced. In Fig. 2, the number of pustules in each treatment is: a, 15 to 20; b, 12 to 15; c, 5 to 7; d, 1 to 2; and E, zero. In each case, when pustules first appear, regardless of their frequency and time of appearance, the value of the RNase specific activity ratio is between 2.4 and 2.7 (Fig. 2). These results show that the appearance of late RNase accompanies the resumption of the growth of the pathogen, but that the quantitative increase in RNase is not a function of the amount of rust mycelium present in the infected leaf.

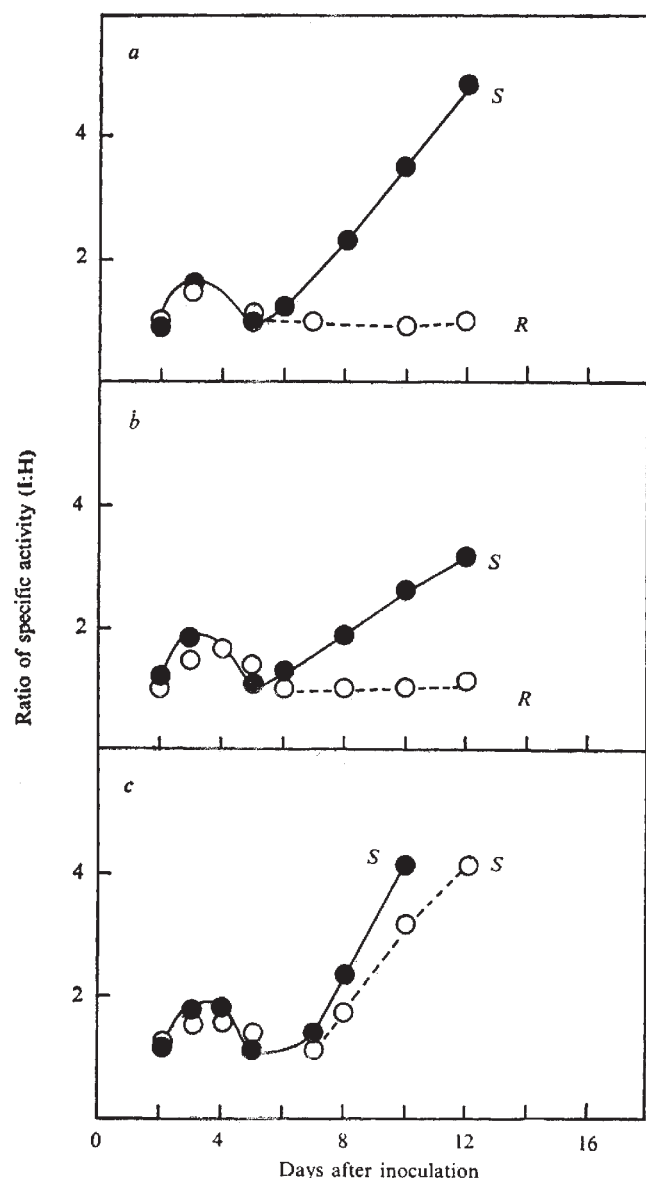


Fig. 1 The effect of temperature regimes on quantitative changes in the RNase activity of the near-isogenic lines of wheat carrying the *Sr6* and the *sr6* alleles following inoculation with wheat rust races 56, 56A and 126 ANZ-6, 7. RNase was extracted from healthy and inoculated primary leaves at 0 to 4° C. Approximately 5 g of leaves were ground in a prechilled mortar with equal amounts (on a weight basis) of sand and polyvinylpyrrolidone (Sigma Chemical Co., St. Louis, Missouri). This was extracted with 15 ml of 40 mM potassium phosphate buffer, pH 6.7, and centrifuged at 10,000g for 30 min. The supernatant fraction was acidified to pH 5.0 with 1 N HCl and left at 0 to 4° C overnight. The precipitate formed during this interval was removed by centrifugation at 10,000g for 15 min. The supernatant was used for the estimation of RNase activity. The reaction mixture for RNase assay contained (final volume 1 ml) sodium acetate buffer, pH 5.0, 50 μ mole; yeast RNA (high molecular weight), 0.8 mg and RNase (15 to 25 μ g protein). Duplicate samples were run without the enzyme. All samples were incubated at 37°C for 30 min, chilled quickly and 2 ml of precipitating reagent (1N HCl in 76% ethanol containing 0.5% LaCl_3) was added to each sample. The content of the tubes was mixed thoroughly, allowed to stand for 10 min and centrifuged until the supernatant was clear. The A_{260} of the supernatant was read against similarly diluted precipitating reagent and corrections were made for the enzyme blanks. One unit of RNase is defined as the quantity of enzyme catalyzing an increase in A_{260} of 1.0 in the standard conditions of assay. Specific activity is units per mg protein. S, Susceptible; R, resistant. a, 20°C; ●, *Sr6*-race 56; ○, *Sr6*-race 56. b, 20°C; ●, *Sr6*-race 56A; ○, *Sr6*-race 126-ANZ-6, 7. c, 26°C; ●, *Sr6*-race 56; ○, *Sr6*-race 126-ANZ-6, 7.

TABLE 1 The substrate preference of RNases from healthy and rust-infected *Sr6* and from axenically-grown rust mycelium.

Source of RNase	Growth temperature	Relative rates of hydrolysis (d.p.m. solubilised per mg protein $\times 10^{-4}$)		
		Poly (A)	Poly (U)	Poly (C)
<i>Sr6</i> (Healthy)	26°C	130	195	75
<i>Sr6</i> (10 ds after inoculation with race 126 ANZ-6, 7)	26°C	289	360	430
Rust mycelium (race 126 ANZ-6, 7)	17°C	267	200	265

Primary cultures of the Australian isolate of wheat rust race 126 ANZ-6, 7 were grown on a chemically defined liquid medium containing, the following (in g l⁻¹): glucose, 3.0; NaNO₃, 2; KH₂PO₄, 1; MgSO₄·7H₂O, 0.5; KCl, 0.5; FeSO₄·7H₂O, 0.01; glutamine, 6.1; cysteine, 0.36; Ca(NO₃)₂·4H₂O, 2 and sodium citrate, 1.5 at pH 5.5. About 2 g of rust mycelium was extracted as described in the legend of Fig. 1. The homopolymers of adenylic, uridylic and cytidylic acids (potassium salt, specific activity 20 μ Ci/ μ mol P) were purchased from Miles Laboratories, Inc., Elkhart, Indiana). The reaction mixtures for the radioassay were the same as those described in the legend to Fig. 1 except that the ³H-labelled homoribopolymers were used instead of yeast RNA. At the end of the incubation period, 0.3 ml of non-radioactive yeast RNA (3.0 mg ml⁻¹) was added to each tube as a coprecipitant and mixed thoroughly before the addition of the precipitating reagent. After centrifugation, an aliquot of the supernatant was processed⁹ for the estimation of radioactivity in a Nuclear Chicago (Mark I) liquid scintillation spectrometer. The c.p.m. of the samples were obtained by the channels ratio counting procedure and the efficiency of counting was determined using a quench correction curve prepared with standard ³H-labelled samples. The radioactivity (d.p.m.) was calculated from these data.

The results in Table 1 show that the RNase from rust infected wheat leaves hydrolyses poly(A), poly(U) and poly(C) at rates 2.2, 1.8 and 5.7 fold those of the enzyme from corresponding uninfected leaves. In addition the substrate preferences of the RNase from uninfected leaves, infected leaves and rust mycelium grown in axenic culture are all different and are, poly(U) > poly(A) > poly(C); poly(C) > poly(U) > poly(A) and poly(A) > poly(C) > poly(U) respectively. Similar changes in the substrate preference of the RNase in flax cotyledons inoculated with *Melampsora lini* and in *Ribes* leaves and cortical tissue of pine inoculated with the diploid and haploid phases of *Cronartium ribicola*, respectively, have been reported elsewhere (ref. 1 and A. E. Harvey, A. K. C., M. S., and L. A. S., unpublished). The results presented thus far show that there is a direct correlation between the ability of the rust fungus to grow in host tissue and the appearance of late RNase (Figs 1 and 2), which has a substrate preference different from the RNases of uninfected host tissue and rust mycelium grown in axenic culture. They suggest that RNase molecules with unique catalytic properties may be required for growth of the pathogen in the host. If this idea has any validity it should, in principle, be possible to detect changes in the properties of RNase in the initial stages of growth of the pathogen in the host.

A series of experiments was therefore carried out in order to investigate this possibility (Table 2). Healthy and inoculated (race 56) *Sr6* plants were maintained at 20° C for 13 d (Fig. 2d). In these conditions, growth of the rust is initiated but is restricted to a few intercellular hyphae, as described earlier. On the fourteenth day, batches of healthy and inoculated plants were transferred to 26° C. Changes

in RNase levels, heat stability and substrate specificities were measured at 3, 6, 10, 13 and 21 d after inoculation (that is 7 d after transfer from 20° C to 26° C. Table 2). For comparison, similar measurements were also made using plants maintained continuously at 26° C for 10 d after inoculation (Table 2). As expected, late RNase does not appear until after the plants are transferred to 26° C. The heat stability (at 80° C for 10 min; see legend to Table 2) of the RNase from healthy leaves varies between 60 and 70%, regardless of the temperature at which the plants are held. The stability of the enzyme from infected tissue is consistently lower, and varies from 52% 3 d after inoculation to 31% at 13 d in plants held at 20° C (Table 2). When late RNase appears at 26° C, its heat stability is

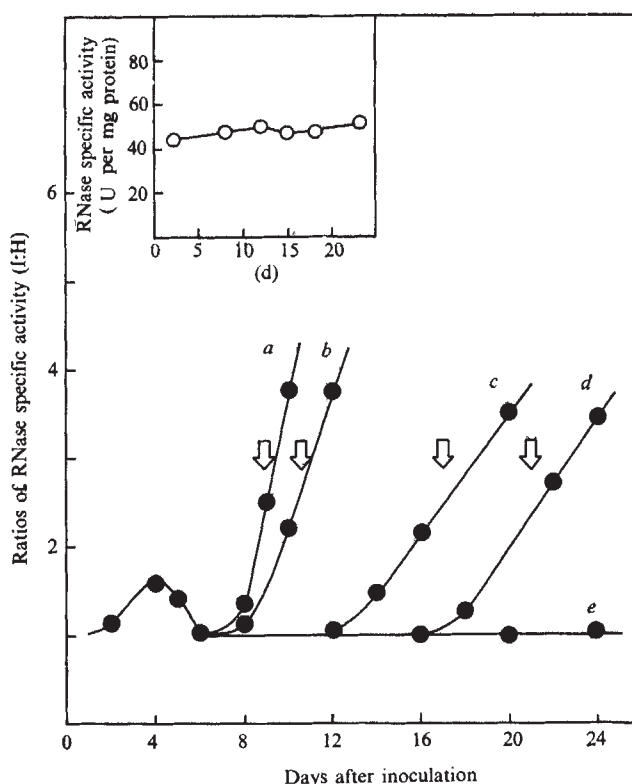


FIG. 2 Changes in the RNase specific activity of wheat plants carrying the *Sr6* allele inoculated with wheat rust race 56 and transferred to 26° C at various times after inoculation. a-e, Five groups of healthy and inoculated plants were maintained at 20° C for different times before they were transferred to 26° C. The plants of group a were transferred 12 h after inoculation and those of b, c and d at 24 h, 5 d and 13 d after inoculation respectively. e, Healthy and inoculated plants never transferred to 26° C. Arrows indicate the approximate time when pustules become visible. The inset shows the specific activities (units per mg protein) of extractable RNase of the primary leaves of healthy plants of group, d, at various intervals beginning with the time when other plants of the same group were inoculated.

even lower (22 to 23%, Table 2). Changes in the heat stability of RNase thus precede the appearance of late RNase.

The relative rates of hydrolysis of poly(A), poly(U), and poly(C) also show striking changes as early as 3 d after inoculation in plants held at 20° C (Table 2). Poly(C) hydrolysing activity increases 3 fold on the third day after

TABLE 2 Effect of temperature regimes on *a*, quantitative and qualitative changes in RNase activity and *b*, intensity of infection in the wheat variety carrying the *Sr6* allele following inoculation with wheat rust race 56.)

Days after Inoculation	<i>Sr6</i> race 56, at 20° C until day 14, then transferred to 26° C						<i>Sr6</i> race 56 at 26° C
	3	6	10	13	21	10	
1 RNase specific activity ratios (I:H)	1.50 (Early RNase)	1.08 (Early RNase disappearing)	0.96 (No late RNase)	1.02 (No late RNase)	2.30 (Late RNase)	3.7 (Late RNase)	
2 Heat stability (% of original activity retained, 10 min at 80° C).	H 63 Inoc 52	70 42	61 32	64 31	61 22	60 23	
3 Relative rates of hydrolysis (Inoc/H) of:							
Poly (A)	1.26	1.49	1.46	1.85	3.70	2.60	
Poly (U)	1.21	0.80	0.90	1.07	3.60	2.10	
Poly (C)	3.16	1.27	1.02	1.04	4.10	3.90	
4 Intensity of infection	No visible symptoms	No visible symptoms	No visible symptoms	No visible symptoms	1 or 2 small pustules per leaf	15–20 pustules per leaf	

The primary leaves of *Sr6* wheat were inoculated with wheat rust uredospores (race 56) and maintained at 20° C. 14 d after inoculation, batches of healthy and inoculated plants were transferred to 26° C. 'Early RNase' and 'late RNase' refer to the early and the later phases of increase in the RNase specific activity ratios (inoculated:healthy) (I:H) respectively, as shown in Fig. 1. Heat stability of RNase was estimated by the following procedure¹. The pH of the RNase solution was raised to 8.5 by adding 1 M NaOH and an aliquot of the enzyme was held at 80° C for 10 min, cooled to 0° C, the pH readjusted to 5.0 with 1 N HCl. The activity of the enzyme was estimated and the loss of activity due to heating at 80° C was determined. The results are presented as the percentage of original activity retained at 80° C. The relative rates of hydrolysis of ³H-labelled poly(A), poly(U) and poly(C) were determined as described in Table 1. The ratios of the rate of hydrolysis (d.p.m. solubilised per mg protein) of the RNase from inoculated leaves to that of the enzyme from healthy leaves for each polynucleotide are presented.

inoculation. Poly(A) hydrolysing activity increases by 26, 49, 46 and 85% on the third, sixth, tenth and thirteenth days after inoculation; after transfer to 26° C and the appearance of late RNase 21 d after inoculation, it increases by more than 3 fold (Table 2). The changes in poly(C) and poly(U) hydrolysing activities appear to parallel total RNase activity; they increase when early RNase appears, decrease when it declines and increase again by 3 to 4 fold after the plants are transferred to 26° C and late RNase appears. These results show that in infected leaves, changes in heat stability and substrate preference of the enzyme precede the increase in activity that we have called late RNase.

Sporulating pustules developed at only one or two infection sites per leaf on the *Sr6* plants held first at 20° C for 13 d and then at 26° C for 7 d. On the other hand, when *Sr6* plants were held at 26° C for 10 d immediately following inoculation, fifteen to twenty type 3 to 4 pustules developed on each leaf. Comparison of the decreases in thermal stability and increases in the rates of polynucleotide hydrolysis by the late RNase from these two samples (Table 2), strongly indicates that the changes in the properties of the enzyme are not directly related to the number and size of the sporulating pustules that finally develop. It is, however, important to remember that each leaf was inoculated with uredospores over its entire surface so that the number of incipient infections is high (at least fifteen to twenty) in each case.

The results presented here show that infection of wheat with wheat stem rust is accompanied by an increase in early RNase, which reaches its maximum activity at 3 or 4 d after inoculation and then declines. In susceptible combinations, the decline in early RNase is followed by a marked rise in late RNase. The appearance of late RNase is correlated with the ability of the pathogen to grow in the host tissue, but not with the number and size of the pustules that finally develop. Increases in the activity of both early and late RNases are characterised by major differences between the heat stabilities and substrate preferences of these enzymes and those of the RNases of the uninfected host and rust mycelium grown in axenic culture. Such qualitative changes in RNase activity are detectable within 3 d after

inoculation. In principle they may well be initiated in the very first stages of host-parasite interaction and may play essential roles in the post transcriptional processing of RNA during the host parasite interactions in both susceptible and resistant combinations. This suggestion is supported by the observation that in rust infected wheat⁷ and flax⁸ increases in RNase activity are accompanied by increased RNA synthesis, which in a rust-infected susceptible variety of flax, involves the preferential synthesis of one or more molecular species of RNA with a high A + U/G + C ratio⁸.

We thank Mr A. Bose for providing the axenic cultures of wheat rust and Dr J. M. Daly, University of Nebraska, for a gift of seed of the near-isogenic lines of wheat. This work was supported by grants (to M.S.) from the National Research Council of Canada and the Canada Department of Agriculture.

Note added in proof: The unpublished paper by A. K. Chakravorty, M. Shaw and L. A. Scrubb and A. E. Harvey, A. K. Chakravorty, M. Shaw and L. A. Scrubb are now 'in the press' *Physiological Plant Pathology*.

ARUN K. CHAKRAVORTY
MICHAEL SHAW
LEROY A. SCRUBB

Department of Plant Science,
Faculty of Agricultural Sciences,
University of British Columbia,
Vancouver 8, BC

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book reviews

Medicine in China

Medicine and Public Health in the People's Republic of China. Edited by Joseph R. Quinn. Pp. xii+333. US Department of Health, Education and Welfare, National Institutes of Health: Bethesda, Maryland, 1973.)

THIS is not a very satisfactory book on three grounds. First, it suffers from the usual disadvantage of a multi-authored book in which no editorial attempt has been made to avoid redundancies and overlap. Second, although the title implies an up-to-date coverage of the subject, this is really only true of two or three out of the fourteen chapters. In fact, of the fourteen authors only two (Dr and Mrs Seidel) wrote out of personal knowledge based on recent visits to the People's Republic of China (PRC). At the time of publication (June 1972 for preliminary soft cover edition) none of the other authors seemed to have visited the PRC during the preceding two decades. Third, it is simply not clear for whom this book was envisaged in spite of the introductory statement that "inasmuch as the audience for this document is intended to be those persons with a general interest in the subject of China medicine rather than the biomedical specialist, the lack of confirming evidence by on-site observation or experimentation was not considered a deterrent to the preparation of this document". This may not have been a deterrent for the authors, but it is likely to deter many readers.

I myself had been unfamiliar with the state of medicine and public health in the PRC prior to a lecture visit in 1973. In preparation for that visit I had skimmed over this book, but had read carefully only two chapters ("Pharmacology" by J. Y. P. Chen and "Population Dynamics" by L. A. Orleans) because they were directly related to my own interests. Only Orleans's chapter proved to be instructive. Upon my return, I reread carefully the entire book and reached the conclusions that as a primer for prospective visitors to the PRC, no more than a third of it is relevant to the present scene and reasonably up to date. The rest is history and much of it so superficial that its main utility is the list of references at the end of most chapters. Specific mention of a few chapters will suffice to illustrate the pros and cons of this book.

R. C. Croizier's chapter on "Traditional Medicine as a Basis for Chinese Medical Practice" offers a brief but reasonably good background to why traditional Chinese medicine with its theoretical basis should be distinguished from ordinary folk medicine. Croizier also explains why this was retained by the Communist regime and integrated into their current medical system together with Western medicine: "After all, if an engineer could learn from a coolie, and an agronomist from an old peasant, why not have a modern medical specialist learn from a native herbalist?" The brief description of the dual forces of the universe, *yin* and *yang*, together with the vital life force, *ch'i*,—though necessary to an understanding of traditional Chinese medicine—is repeated in three other chapters. Even a minimal editorial effort could have avoided some of this duplication. Perhaps the most appropriate place for a discussion of this philosophical basis of Chinese medicine is the longest chapter of the book, "Acupuncture" by J. Y. P. Chen. Although its length is appropriate in view of the importance of acupuncture in China, both past and present, it overdoes the detailed operational aspects at the expense of an adequate discussion of its theoretical basis and of current work on the mode of action of acupuncture anaesthesia.

The other chapter by Chen entitled "Pharmacology" is based primarily on a perusal of Chinese journals published until 1967 and subsequently upon newspaper accounts. Its list of plant and animal drugs ranges from the superficial to the ridiculous (for example, grasshoppers and locusts "have the effect of calming the nerves and stopping coughs and they are used in whooping coughs and tetanus. Grasshoppers are said to be specific for cases of pertussis and asthma"). By contrast, China's modern pharmaceutical industry rates only one page. Yet this is one of the most impressive achievements of the PRC. Few Western visitors are prepared for the total self sufficiency of the PRC in terms of modern drugs—ranging from all currently used antibiotics to steroid oral contraceptives which are synthesised on a scale which is hardly equalled in the United States. No mention is made of the first class work on the isolation and chemical characterisation of traditional Chinese herbal medicines at the Institute of Materia Medica in Peking. Nowhere in this chapter, or in-

deed anywhere else in this book is there any description of the rather impressive labelling practice in the PRC—each drug container listing the date of manufacture, name of factory, name (in Latin for "Western Medicines") of drug, quantitative analysis, and so on. In fact, V. Seidel's chapter on "Medical Personnel and their Training" with his table (pages 169–171) listing the contents of a "barefoot doctor's" bag is much more instructive in giving the reader a view of the state of today's drug development in the PRC.

By far the most readable chapter in the entire book is the one on "Population Dynamics" by L. A. Orleans, which deals in an authoritative way with demographic aspects, family planning programmes and public health. What is not clear to the uninitiated reader is that it reflects accurately the state of affairs up to the Cultural Revolution, but that much has changed in the PRC during the past five years in the field of birth control (see C. Djerassi, *China Quarterly*, **57**; 1974). Curiously enough, this best chapter is the only one which lacks references.

The price of this book is not indicated but is likely to be relatively cheap since it is published by the US Department of Health, Education and Welfare. Nevertheless I am hard pressed to guess for whose library it should be purchased.

CARL DJERASSI

Rare earth magnets

Rare Earth Intermetallics. By W. E. Wallace. (Materials Science and Technology.) Pp. xii+266. (Academic, (Harcourt Brace Jovanovich): New York and London, September 1973.) \$22.50.

THE extraordinary magnetic properties of the rare earth metals—which are not really rare—have only been appreciated during recent years, and the time is ripe for a concise appraisal. Herein lies the great value of this monograph which provides a fascinating, orderly and critical presentation of the properties of rare earth intermetallic compounds, collated up to the summer of 1971.

The authors give a useful, short table of symbols to describe the main types of magnetic order and paramagnetic behaviour. They discuss types of magnetic interaction and the Rudermann-Kittel-Kasuya-Yoshida concept of indirect exchange interaction between rare earth ions through conduction elec-

trons, and the de Gennes contributions to the theory. A chapter on (electrostatic) field interaction and its effect on magnetic and thermal behaviour of rare compounds pays tribute to the pioneer work of Penney and Schlapp, Bleaney and Stevens, and Van Vleck which helps us to explain so much of this work. A helpful illustration is given of the use of the Stevens operator equivalent method of dealing with the magnetic and thermal effects of the crystalline field on rare earth ions, but it is difficult to understand the necessity or wisdom of including in this book an appendix of forty-eight very closely packed pages of numerical data on energies, eigenfunctions, and so on of rare earth ions in a hexagonal field.

I much appreciated the orderly layout of the five chapters on rare earth-non-transition metal systems, and of the four chapters on rare earth-transition metal systems in which Ni, Co, Fe and Mn loom large, each with its wealth of tables of magnetic characteristics and graphs of magnetic and thermal behaviour. I was also interested in the many references to the Kondo effect, and to Mössbauer and neutron diffraction experiments. Much discussion is given to phenomena which are associated with the filling of the *d* bands in the transition metals—the variation of the magnetic moment of cobalt and iron with temperature. A brief treatment is given of the use of rare earth materials for permanent magnets, where the rare earth is thought to provide a uniaxial crystal with high Curie point, large saturation magnetisation and strong anisotropy. The commercial evaluation of the RCo₅ system is in its infancy; the RFe systems do not seem encouraging, but perhaps RFeCo systems may later be found to be so.

This is an excellent monograph; though it would be improved by the addition of a subject index.

L. F. BATES

Inorganic initiation

Inorganic Chemistry: Principles of Structure and Reactivity. By James E. Huheey. Pp. xvi+737. (Harper and Row: New York, Evanston, San Francisco and London, 1972.) £3.85.

THIS is an interesting, original and stimulating book but a difficult one to place: it certainly cannot be regarded as a comprehensive text on inorganic chemistry. Its main aim seems to be to introduce the university student to the fascination of research publications in many areas of modern inorganic chemistry: it starts typically with perbromate on page 1 and xenon tetroxide on page 2.

The first two thirds of the book are concerned with the development and

application of principles, the emphasis throughout being on structure and bonding in molecular compounds and complexes. Solid state chemistry and thermodynamics are virtually absent, but there is a very full discussion of the many attempts to rationalise covalent bonding in inorganic systems. The last third is a series of essays on such topics as organometallic chemistry; chains, rings and cages; noble-gas and positive-halogen chemistry; lanthanides and actinides; and biological systems.

The book is very easy to read, full of delightful touches, and clearly the work of an enthusiastic teacher. It is well illustrated and has excellent references to further readings on a wide variety of subjects. It will surely appeal to many students and it is excellent value.

C. S. G. PHILLIPS

This earthly life

Life on Earth. By Edward O. Wilson *et al.* Pp. xi + 1033. (Sinauer: Stamford, Conn; Freeman: Reading, September 1973.) £5.40.

THIS college biology textbook claims to "pioneer a new generation" of such publications. If it is not, perhaps, such a complete innovation as this, it is, nevertheless, a good basic book which has some unique features.

A publication which boasts eight authors runs the risk of presenting a divisive view of its subject, but the quintessence of modern biology offered here escapes this hazard. Although the sections differ in style and approach, these differences merely reflect the intrinsic differences in their themes and a sound system of indexing and cross reference helps to unify the whole.

It starts with a kind of prologue, "Life on the Third Planet". There is an opening quotation from James A. Lovell who saw the earth, as he returned to it in Apollo 8, as "the only place that had color, the only place that had life . . . that had warmth . . . that was home to us". The first of the five main sections, "The Cell", covers, in about one quarter of the book, the structure of the cell, biological reactions, enzymes, energy, codes and genes, genetics and the control of cell chemistry. Section 2, "Multicellular Life", again comprises about one quarter of the whole and includes chapters on: development, reproduction, carbon and nitrogen sources, gas exchange, transport systems, the internal environment, neurobiology, effectors, behaviour instinct and ethology, and the modification of behaviour. Section 3 describes the diversity of life in a traditional way and Section 4, "The Strategy of Evolution", includes chapters on biogeography, the growth and

interaction of populations, ecosystems, social behaviour and human evolution. The last section, "Alternate Futures", presents contrasting optimistic and pessimistic prophecies.

In all, this is a comprehensive, exciting and intellectually challenging book which throws illuminating sidelights on the personalities and work of biologists, on the use of biological materials in everyday concerns and on the significance of biological processes for mankind. The format is pleasing, if fragmentary, there are many striking photographs, and explanatory diagrams are well correlated with the text. Controversial issues are developed in parenthetical, colour-panel insertions so that they do not interrupt the development of the main thesis. A list of related readings from specialised books, articles and research papers is given at the end of each chapter. There is a glossary of biological terms. *Life on Earth* would certainly be a useful volume in a school or college library and might serve as a basic, if weighty, sixth form or college text.

E. J. VINNICOMBE

Interface chemistry

An Introduction to the Principles of Surface Chemistry. Edited by R. Aveyard and D. A. Haydon. (Cambridge Chemistry Texts.) Pp. xvi+232. (Cambridge University: London, August 1973.) £5.40 cloth; £2.40 paper.

THIS book is a must for all surface chemists, but particularly those meeting the subject for the first time, either in formal course work or starting research. I also strongly recommend it for all those teaching the subject as it is one of the clearest and most logical expositions of the physical chemistry of interfaces I have encountered. It assumes the reader is familiar with the basic thermodynamics and statistical mechanics met in most undergraduate courses, and on this foundation builds the subject up to a level where the reader can take the subject further by reading some of the heavier texts or recent review articles referred to in the book. It is refreshing to see a limited number of carefully selected, relevant references referred to in the text, rather than the seemingly endless list, bewildering to the beginner, normally encountered.

The authors do not exceed the aim set in the book's title. It does not attempt to deal in depth with experimental aspects, or applications, of the subject, thus leaving room (unlike many other text books in the field) to give full derivations of many of the more important equations. In short, this book fills a gap in the literature and is well worth the price.

B. VINCENT

broadcast science

Radio components

ON Saturday afternoons, BBC Radio 4 has recently been providing a "Dial a Scientist" programme which gives a genuine insight into what the radio customer wants, in scientific terms. Questions asked recently include:

- Why do stars twinkle?
- Why is water wet?
- Why do African elephants have larger ears than do Indian elephants?
- Why does the hair of babies change colour?
- What is the twin paradox?
- Why has the Earth not cooled down?

This kind of programme should be compulsory listening for all producers of science programmes. There clearly is a broad interest in science, which BBC Radio is still not catering for satisfactorily. In particular, perhaps it is not too late for "Kaleidoscope" to live up to its description as Radio 4's nightly review of the arts and sciences. The only thing wrong with the science on "Kaleidoscope" is that often one feels the need of a microscope to find any; perhaps the recent arrival of "Scientifically Speaking" (see *Nature* 247, 411; 1974 for a review) will be taken as a spur to show that, at least in the 'science is fun' department, "Kaleidoscope" can do for the layman what John Maddox

seems to be trying to do for the professional scientist.

Welcome surprise

David Davies

INDEPENDENT Television served up an unexpected offering on January 31—half an hour on anorexia nervosa—and on this showing they should chance their arm more often on science and medicine. Certainly there are enough diseases to keep British viewers fascinated for a whole season.

The programme was written, produced and directed by Robin Brown and it was a brave job, although one suspects he could have done with a bigger budget. As it was the presentation fell between two stools. The disease, a compulsive teenage refusal to eat adequately, was clearly described early on, and this would have made an excellent fifteen minute documentary, one of a series. But Mr Brown's imagination, or pocket, did not stretch to half an hour and so things got thinner as time went on. With such a length of programme it is necessary to go into some detail—is it a complaint of the well-off only, how many actually die of it, how many later lead a perfectly normal life, how does it divide amongst the

sexes? One hardly got the impression that it had been difficult cramming everything in.

Moreover, there was a strange air of unreality to many of the scenes. If viewers can take starving Ethiopians, they can presumably also take over-shm English girls, yet none of those shown looked in any great distress; in fact they all seemed to have got better before being filmed. Two minutes with someone visibly suffering would have got the story over very effectively. This lack of verisimilitude was compounded by stilted dialogue.

There was, however, one quite extraordinary moment. Anorexia nervosa is a complaint which tends to involve and confuse the family and generate strange family situations in which powerful forces are abroad but are manifested in odd material ways. A mother was filmed reminiscing with her daughter about spraying wash basins and lavatory bowls with red ink to attempt to dissuade her from being sick in them and having to wash away the tell-tale markers. This was a stunning insight into the dilemma of the family and produced a chilly moment.

We were mercifully spared commercials till the end when we were exhorted to consume baby foods, thick soups and juicy oranges.

obituary

A. S. Romer

Dr Alfred Sherwood Romer, Emeritus Professor at Harvard University, who died on November 5, aged 78, became one of the most outstanding names in the history of Vertebrate Palaeontology. Graduating at Amherst he moved to Columbia University where he worked in the school of Professor W. K. Gregory and was first concerned with the leg musculature of dinosaurs. In 1923 he was called to the University of Chicago and here his major work was on the Pelycosauria. After many years of field work and laboratory study, and after publishing a series of papers, he wrote with L. I. Price, a brilliant artist, a mon-

ograph on these animals published in 1940, which is a model for such work. He also published valuable papers on other early reptiles and the amphibia, and he wrote a monograph on the labyrinthodonts in 1947 which will long remain the standard work on these animals.

In 1937 Romer sectioned the head of the fish *Ectosteorhachis* and, as a result, he immediately recognised the correctness of Westoll's solution to the problem of fish dermal bone nomenclature. He was a leading champion of the view that vertebrates evolved in fresh water. This reflects the constant attention he paid to the geological background of the animals he studied, work which resulted in a sound knowledge of the continental deposits of the Lower Permian of Texas

on which he became a recognised authority.

Romer discussed usefully such troublesome matters as the nature of *Diadectes*, the origin of the Ichthyosauria, and the relationships of *Araucoscelis* and of the so-called Microsauria. It was Romer who realised that the phyllospindylous amphibia were not an independent order, as had long been held, but only the larvae of labyrinthodonts as was originally believed. He questioned the view that the rhachitinous vertebrae of labyrinthodonts were derived from the embolomorous type and was eventually able to demonstrate that the reverse was, in fact, true.

At an age when most palaeontologists have long ceased from active field work

Al Romer was making discoveries of new faunas in South America. This entailed hard physical exertions and resulted in the discovery of extremely important Middle Triassic faunas (missing from the classical South African Karroo) including both synapsid reptiles, long needed to illustrate fully the origin of mammals, and of new early archosaurs. These explorations resulted in a series of clearly written papers, showing the balanced judgement which characterised Romer's writings throughout his long working life.

One of Romer's greatest calls to fame lies in his text books. His *Vertebrate Palaeontology*, published in 1933 when no other such work existed in the English language, was an immediate success. It surveyed the whole field of vertebrate palaeontology with balance and judgement and was excellently illustrated and it was of the greatest help to all students of the subject. The accompanying *Man and the Vertebrates*, of a lesser standard, was intended to protect the publishers against a loss on the main work—which never occurred. His famous book was republished in 1945 and was almost entirely rewritten and reillustrated again by the author in 1966, thirty three years after the publication of the first edition! He also published in 1949 a book on the comparative anatomy of the vertebrates, called *The Vertebrate Body*, of great value to students, dealing with the embryology, osteology and dentitions (he once remarked "I hate teeth"), the circulatory system, the CNS, the myology and the digestive systems. Not content with that he published in 1956 his famous *Osteology of the Reptiles*, to replace the largely out of date work by Williston. Here, as in his *Vertebrate Palaeontology*, his name will long remain a familiar one on the shelves of zoological libraries. His last book, *The Procession of Life*, was published in English in 1968; it was published also in French, German and Italian and these Continental editions are superbly illustrated with many coloured plates.

Romer became Director of the Museum of Zoology at Harvard in 1946, having been Professor of Zoology and Curator of Vertebrate Palaeontology since 1934. He was Alexander Agassiz Professor of Zoology from 1947 until 1965 and was then elected Emeritus Professor. He was held in the greatest respect all around the world, which he travelled extensively, and was honoured by the award of an immense number of medals and honorary degrees. He was a Member of the Academy of Sciences, a Past President of the American Association for the Advancement of Science a Foreign Member of the Royal Society and an honorary member of many others.

When his research work, his teaching and his books are considered together

his contribution to vertebrate zoology can only be described as formidable.

A host of friends were indebted to him and his charming wife for their never failing help, kindness and hospitality.

Announcements

Appointments

P. C. Elwood has been appointed **Director of the Medical Research Council Epidemiology Unit** (South Wales).

Professor J. Heslop-Harrison, FRS, Director of the Royal Botanic Gardens, Kew, has been elected **President of the Institute of Biology** for 1974.

Professor J. H. Beynon, senior research associate at ICI and professor of chemistry at Purdue University, Lafayette, has been appointed to a **Royal Society Research Professorship** at the **University College of Swansea, University of Wales**.

Sir Owen Saunders, FRS, Emeritus Professor of Mechanical Engineering at Imperial College, University of London, has been appointed **Royal Society Visiting Professor** at the **Engineering School of the Federal University of Rio de Janeiro**.

Awards

The first **Tribology Bronze Medals** of the **Institution of Mechanical Engineers** have been awarded to **D. R. Adams, D. R. Garner and R. A. Onions**.

Aslib (formerly the Association of Special Libraries and Information Bureaux) is to mark its 50th anniversary in 1974 by the award of a prize of £250 and a piece of engraved silver for the best paper submitted in competition on *The Future Pattern of Information Services for Industry and Commerce*.

International Meetings

March 1, **Biodegradation of Town Refuse and Farm Wastes** (Marian Honer, Assistant Information Officer, University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET)

March 4, **Catalysis and Inhibition of Oxidation Processes** (Dr R. F. Greenwood, Hon. Secretary, London Section, Society of Chemical Industry, Chemistry Department, The City University, St John Street, London EC 1V 4PB)

March 4-6, **Interim Meeting of the Society of Clinical Pathologists and the College of American Pathologists** (Steven K. Herlitz, Inc., 850 Third Avenue, New York, N.Y. 10022)

March 4-8, **25th Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy** (Robert W. Baudoux, Exposition Chairman, U.S. Steel Corporation, Research Laboratory, M.S. 57, Monroeville, Pennsylvania 15146)

March 5-6, **Noise Measurement and Control** (Course Organiser, Birniehill Institute, National Engineering Laboratory, East Kilbride, Glasgow G75 0QU)

March 6, **Meeting of the Particle Size Analysis Group of the Society for Analytical Chemistry** (Analytical Division, Chemical Society 9/10 Savile Row, London W1X 1AF)

March 6-8, **Minicomputers: Fundamentals and Applications** (Lisa Spaducci, Polytechnic of Central London, 115, New Cavendish Street, London W1M 8JS)

March 7, **Component Interactions in Fluid Flow Systems** (A. J. Tugwell, 1, Birdcage Walk, Westminster, London, SW1H 9JJ)

March 11-15, **Chemical Society Management Studies for Chemists** (Dr M. D. Robinson, The Chemical Society, Burlington House, London W1V 0BN)

March 13, **Prediction of Geological Hazards** (Professor B. M. Funell, Environmental Sciences, University of East Anglia, Norwich NOR 88C)

March 14-15, **Heat Pipes** (The Short Course Unit, The Polytechnic of Central London, 35, Marylebone Road, London NW1 5LS)

March 18, **Microcalorimetry in Biology** (The Assistant Secretary, Society of Chemical Industry, 14, Belgrave Square London SW1X 8PS)

March 18-22, **6th Semi-Annual Short Course on Laser Safety** (Laser Safety Course, CONMED, 114 Medical College, Cincinnati, Ohio 45229)

March 19, **Group Technology: A Method for Improving Productivity** (Course Organiser, Birniehill Institute, National Engineering Laboratory, East Kilbride, Glasgow G75 0QU)

March 21, **General Meeting of the Institution of Mining and Metallurgy** (The Geological Society, Burlington House, Piccadilly, London W1V 0JU)

March 20-21, **Computers in Medicine** (The Secretary, World Organisation of General Systems and Cybernetics, Blackburn College of Technology and Design, Feilden Street, Blackburn BB2 1LH)

March 20-22, **Tetrahedrally Bonded Amorphous Semiconductors** (Marc H. Brodsky, Conference Chairman, IBM Thomas J. Watson Research Center P.O. Box 218, Yorktown Heights, New York 10598)